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Health and Environmental Effects Profile for Nitrotoluenes (o-, m-, p-)

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16. ABSTRACT

The Health and Environmental Effects Profile for Nitrotoluenes (o-, m-, p-) was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Nitrotoluenes has been determined to be a systemic toxicant. An Acceptable Daily Intake (ADI), defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect, for nitrotoluenes is 0.0143 mg/kg/day for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for nitrotoluenes is 1000.

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR NITROTOLUENES (o-, m-, p-)

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched."

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include, Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor or q_1^* is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3112. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA docket.

ACKNOWLEDGEMENTS

The initial draft of this report was prepared by Syracuse Research Corporation under Contract No. 68-03-3228 for U.S. EPA's Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH. Dr. Christopher DeRosa was the Technical Project Monitor and Helen Ball was the Project Officer. The final documents in this series were prepared for the Office of Solid Waste, Washington, DC.

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EXECUTIVE SUMMARY

The mononitration of toluene results in the formation of three isomeric nitrotoluenes. o- and m-Nitrotoluenes are clear yellow liquids; p-nitrotoluene is a colorless solid at ambient temperature. The three isomers are slightly soluble in water but are readily soluble in organic solvents (Dunlap, 1981). The air odor threshold for m-nitrotoluene is such that 50-90% of distracted persons perceive warning of its presence at the TLV levels of 2 ppm (Amoore and Hautala, 1983). In aqueous solution, o-nitrotoluene is oxidized with ozone at neutral pH (Munter et al., 1984).

Only two companies manufacture these chemicals in the United States, but the recent production volumes are not available. The total U.S. production volume of the three isomeric nitrotoluenes as of January, 1980, was estimated to be ~44 million pounds. The three nitrotoluenes are manufactured by nitration of toluene with a mixture of nitric acid and sulfuric acid, yielding primarily o- and p-nitrotoluene. The major use for the nitrotoluenes is in the manufacture of intermediates for azo and sulfur dyes (Dunlap, 1981).

The atmospheric fate of nitrotoluenes has not been studied comprehensively. Significant removal of nitrotoluenes from the atmosphere will probably occur because of its reaction with NO_x and sunlight (Nojima and Kanno, 1977); however, the half-life for this reaction cannot be estimated because of the unavailability of reaction rate data. Significant removal of nitrotoluenes from the atmosphere through physical removal processes such as wet and dry deposition is not likely. The data base for nitrotoluenes is inadequate for accurate prediction of their atmospheric fates and half-lives.

Humus-sensitized photoreaction of nitrotoluenes may remove significant amounts of these compounds from natural waters. The estimated half-life for this reaction is 3 days (Zepp et al., 1984). The removal of nitrotoluenes from most natural waters through biodegradation may not be significant (Alexander and Lustigman, 1966). Significant biodegradation of nitrotoluenes will occur under sewage treatment conditions with acclimatized activated sludge (Pitter, 1976; Hallas and Alexander, 1983), although the Japanese MITI test lists these compounds as degradation resistant (Kawasaki, 1980; Kitano, 1978). Significant removal of nitrotoluenes from aquatic media through volatilization is unlikely, but removal of some nitrotoluenes from the aquatic phase by sorption onto suspended particulate matter may occur. The overall half-life of the three nitrotoluenes in river water is estimated to be ~3 days (Zoeteman et al., 1980). Significant bioconcentration of these compounds in aquatic organisms is unlikely (Sasaki, 1978).

Data pertaining to the fate of nitrotoluenes in soil are inadequate. At lower concentrations, nitrotoluenes are not likely to leach from soil to groundwater (Zoeteman et al., 1980; Piet et al., 1981; Piet and Morra, 1983). It has been speculated that a significant loss of nitrotoluenes through volatilization from soil will not occur.

The only available atmospheric monitoring data in the United States reported the presence of o-nitrotoluene and p-nitrotoluene in 2/12 samples collected around a manufacturing facility. The concentrations of o- and p-nitrotoluene in these two samples were reported to range between trace and 86 ng/m^3 (Pellizzari, 1978). Nitrotoluenes have been detected in wastewater from paper mills, TNT production facilities and chemical plants. The concentrations of o- and p-nitrotoluene in wastewater from TNT production facilities may be as high as 16 and 9.2 ppm, respectively (Howard et al., 1976). With recent treatment technologies, however, the level of o- and

p-nitrotoluene in TNT wastewater samples at the 90th percentile concentration may be 0.030 and 0.100 ppm, respectively (Pearson et al., 1979). Nitrotoluenes have been detected in waters of the Rhine River in the Netherlands, with reported concentrations of o-, m- and p-nitrotoluene of 10, 1 and 10 ppb, respectively (Piet and Morra, 1983). Nitrotoluenes have not been detected in drinking water or foods either in the United States or elsewhere in the world.

Although the aquatic toxicities of the nitrotoluene isomers are similar, m-nitrotoluene appears to be the most acutely toxic to fish, algae and bacteria, while p-nitrotoluene is the most toxic to Daphnia. Snails (Lymnaea stagnalis) and Daphnia magna are especially sensitive to the reproductive effects of p-nitrotoluene, with NOECs $\leq$1.0 mg/ℓ. Eggs of the medaka fish, Oryzias latipes, are also sensitive to p-nitrotoluene with a NOEC of 1.0 mg/ℓ for mortality. The blue-green alga, Microcystis aeruginosa, is the aquatic species most sensitive to nitrotoluene toxicity in general, having toxicity thresholds of 1.0-3.3 mg/ℓ for the three isomers.

The nitrotoluenes were absorbed extensively from the gastrointestinal tract of rats following oral dosing (Chism et al., 1984). Absorption following inhalation and dermal exposure can be inferred from the demonstrated toxicity following occupational exposure (Chapter 5). Information regarding the distribution of nitrotoluenes was not available. The data on metabolism indicate that following absorption by the gastrointestinal tract, the nitrotoluenes are oxidized to nitrobenzyl alcohols by microsomal cytochrome P-450 in the liver (Gillette, 1959; deBethizy and Rickert, 1984). Subsequent oxidation to nitrobenzoic acids occurs in the cytosolic fraction of hepatocytes (deBethizy and Rickert, 1984). Metabolism to nitrobenzoic acids appears to be a common process among mammalian species (Chakraborty and Smith, 1967). In rats, conjugation of nitrobenzoic acids with glycine

results in the formation of nitrohippuric acids (Chism et al., 1984). Nitrobenzyl alcohols can also be sulfated and conjugated with glutathione or conjugated with glucuronic acid. Glucuronides are excreted in the bile (or nitrobenzoic acid may be secreted) into the intestine, where they are reduced by intestinal microflora (Long and Rickert, 1983; Doolittle et al., 1983; Chism et al., 1984). The reduced metabolites can be reabsorbed by enterohepatic recirculation into the liver, where a reactive species binds to hepatic macromolecules and damages DNA (Doolittle et al., 1983; Long and Rickert, 1983; Rickert et al., 1984). Quantitative and qualitative differences in these processes for the three isomers in rats result in quantitative and qualitative differences in in vitro metabolites, urinary metabolites and the expression of toxic effects (deBethizy and Rickert, 1984; Chism et al., 1984). Between 80 and 90% of orally administered radioactivity from [^{14}C]-nitrotoluene was excreted in the urine, feces and expired air within 72 hours; the urinary route was the major route (Chism et al., 1984).

Carcinogenicity testing by the National Toxicology Program of p-nitrotoluene by gavage treatment of rats and mice is in progress; the o- and m-isomers are not scheduled for testing (NTP, 1985). No significant differences in the incidence of skin papillomas or carcinomas were observed in mice treated topically with single applications of o-nitrotoluene (24-240 mg/mouse) or p-nitrotoluene (50-400 mg/mouse) followed by 30 weekly applications of TPA as a promoter, when compared with mice treated with TPA alone (Slaga et al., 1985). The response to the p-isomer was considered clearly negative, while the response to the o-isomer was considered suggestive of a weak response. Equivocal results were obtained in a pulmonary adenoma assay, in which mice were injected intraperitoneally with o-nitrotoluene

(840-4800 mg/kg) or p-nitrotoluene (1800-9000 mg/kg), 3 days/week for 8 weeks (Slaga et al., 1985). Therefore, the data are inadequate to conclude that the nitrotoluenes are carcinogenic.

The nitrotoluenes have been tested for mutagenicity and genotoxicity with different responses depending upon the isomer tested. In general, m-nitrotoluene was negative for reverse mutation in Salmonella typhimurium, with and without metabolic activation, and in assays for UDS, chromosome aberrations and SCE in mammalian systems. For p-nitrotoluene, equivocal results were obtained in reverse mutation assays, but positive results were obtained in assays for mitotic gene conversion in Saccharomyces cerevisiae, and SCE and chromosome aberrations in mammalian cells. o-Nitrotoluene was negative for reverse mutation in S. typhimurium, with and without S-9 and for chromosome effects and UDS in vitro; however, Doolittle et al. (1983) presented evidence that intestinal microflora played a role in the bioactivation of o-nitrotoluene to a metabolite capable of damaging liver DNA.

No malformed offspring resulted from matings of male and female rats treated by gavage with 200 mg/kg/day o-nitrotoluene, 300 mg/kg/day m-nitrotoluene or 400 mg/kg/day p-nitrotoluene, 5 days/week for 3 months before mating and throughout gestation (Ciss et al., 1980). Slight histological lesions, however, were observed in the spleens of the offspring of rats exposed to m-nitrotoluene. Reproductive capacity of the adult rats was not impaired at 3 months; however, after 6 months of treatment with p-nitrotoluene, male rats had histological evidence of testicular atrophy and necrosis of seminiferous tubules.

Subchronic studies indicate that exposure to nitrotoluenes resulted in kidney and liver lesions and evidence of hemolytic anemia with resultant

increased erythropoiesis. In the study by Ciss et al. (1980), rats treated with m- or p-nitrotoluene had alopecia, rats treated with any of the isomers had lesions in the spleen and rats treated with o-nitrotoluene had hyalin deposits and lesions in the renal tubules. Treatment of rats with 322 mg/kg/day o-nitrotoluene, 318 mg/kg/day m-nitrotoluene or 392 mg/kg/day p-nitrotoluene daily for 30 days or 3 days/week for 3 months resulted in hematological effects suggestive of hemolytic anemia (Kovalenko, 1973; Vasilenko and Kovalenko, 1975). Sulfhemoglobinemia was a common finding for all isomers and treatment regimens. Methemoglobinemia, reduced hemoglobin concentrations, decreased erythrocyte counts, increased reticulocyte counts, Heinz body formation and prolonged clotting time were observed depending upon the isomer administered and the treatment regimen. Treatment of rats by gavage with a mixture of isomers at 480 mg/kg/day and possibly at 240 mg/kg/day for 1.5-2 months resulted in signs of CNS toxicity immediately after dosing (Kosachevskaya, 1967). The severity of hematological changes increased with dose, and some rats treated with 480 mg/kg/day had hemosiderosis of the spleen and fatty degeneration of the liver.

Occupational exposure to nitrotoluenes has been associated with optic neuropathy (Nemtseev and Smolyaninova, 1976), dysfunction of the adrenal cortex (Makotchenko, 1974) and disturbances of intestinal digestion (Kleiner and Stovpivskaya, 1981). Ingestion of 1 oz to 1 pt of the nitrotoluenes may be fatal to humans (OHM/TADS, 1985).

The acute oral LD_{50} values for the nitrotoluenes in rats range from 890-1610 mg/kg for o-nitrotoluene, 1072-1590 mg/kg for m-nitrotoluene and 1960-2140 mg/kg for p-nitrotoluene (NIOSH, 1985; Vernot et al., 1977; Vasilenko and Kovalenko, 1976).

Pending the results of the carcinogenicity study of p-nitrotoluene by NTP (1985) and more definitive toxicity studies, a provisional ADI of 1 mg/day is recommended to protect a 70-kg human from the toxic effects of all three nitrotoluenes. The ADI was based on a subchronic LOAEL of 143 mg/kg/day (200 mg/kg/day, 5 days/week) of o-nitrotoluene administered to rats and the use of an uncertainty factor of 10,000. The 143 mg/kg/day dose of the o-isomer was associated with splenic lesions in male rats (Ciss et al., 1980).

An RQ of 1000 for chronic oral toxicity of the nitrotoluenes was also derived for the three nitrotoluene isomers and was based on the study of Kovalenko (1973) and Vasilenko and Kovalenko (1976).

The lack of dose-response tumor data for the nitrotoluenes administered by a relevant route precluded the calculation of a potency factor (F), and the nitrotoluenes were placed in IARC Group 3 (IARC, 1982). Based on EPA's proposed guidelines, the nitrotoluenes are classified as Group D, not classified as to human carcinogenicity (U.S. EPA, 1984).

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LIST OF ABBREVIATIONS

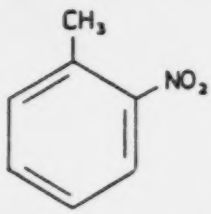
ADI	Acceptable daily intake
BCF	Bioconcentration factor
CAS	Chemical Abstracts Service
CNS	Central nervous system
DNA	Deoxyribonucleic acid
EC_{50}	Concentration effective to 50% of recipients (and all other subscripted concentration levels)
GC/MS	Gas chromatography/mass spectrometry
HPLC	High performance liquid chromatography
K_{oc}	Soil sorption coefficient
K_{ow}	Octanol/water partition coefficient
LC_{50}	Concentration lethal to 50% of recipients (and all other subscripted dosage levels)
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
MED	Minimum effective dose
MTD	Maximum tolerated dose
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NOAEL	No-observed-adverse-effect level
NOEC	No-observed-effect concentration
NOEL	No-observed-effect level
ppm	Parts per million
ppb	Parts per billion
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value

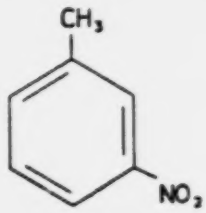
SCE	Sister chromatid exchange
SEM	Standard error of the mean
TLV	Threshold limit value
TNT	Trinitrotoluene
TPA	Tetradecanoylphorbal acetate
TWA	Time-weighted average
UDS	Unscheduled DNA synthesis
v/v	Volume per volume

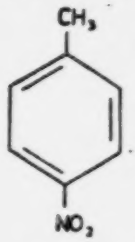
1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

The mono-nitration of toluene results in the formation of three isomeric nitrotoluenes. The structure, common names and synonyms, empirical formula, molecular weight and CAS Registry numbers for these isomers are as follows:







Common name:	o-nitrotoluene	m-nitrotoluene	p-nitrotoluene
Synonyms: (OHM/TADS, 1985)	2-nitrotoluene	3-nitrotoluene	4-nitrotuluene
	1-methyl-2-nitro-benzene	1-methyl-3-nitro-benzene	1-methyl-4-nitro-benzene
	2-methyl-1-nitro-benzene	3-methyl-1-nitro-benzene	4-methyl-1-nitro-benzene
	o-methyl nitro-benzene	m-methyl nitro-benzene	p-methyl nitro-benzene
Empirical formula:	$C_7H_7NO_2$	$C_7H_7NO_2$	$C_7H_7NO_2$
Molecular weight:	137.13	137.13	137.13
CAS Registry number:	88-72-2	99-08-1	99-99-0

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Both o- and m-nitrotoluenes are clear yellow liquids, and p-nitrotoluene is a colorless crystalline solid at room temperature. The three isomers are only slightly soluble in water but are soluble in ethanol, benzene and diethyl ether (Dunlap, 1981). The physical properties of the three isomeric nitrotoluenes are listed in Table 1-1.

TABLE 1-1

Physical and Chemical Properties

Compound:	o-nitro-toluene	m-nitro-toluene	p-nitro-toluene	
Melting point °C:	-9.55 (α form) -3.85 (β form)	16.1	51.7	Dunlap, 1981
Boiling point °C: (at 1 atmos)	221.7	231.9	238.5	Dunlap, 1981
Density d_4^{20}: (g/cm^3)	1.163	1.157	1.286	Dunlap, 1981
Flash point °C: (closed cup)	106	106	106	Dunlap, 1981
Vapor pressure at 25°C: (mm Hg)	0.21	0.20		Hine and Mookerjee, 1975
			0.005	Lenchitz and Velicky, 1970
Water solubility (mg/ℓ): at 30°C	652	498	442	Verschueren, 1983
at 20°C			288	Hashimoto et al., 1984
Seawater at 20°C			251	Hashimoto et al., 1984
Log K_{ow}:	2.30	2.45	2.37	Hansch and Leo, 1985
Estimated Henry's law constant: ($atm \cdot m^3/mol$)	5.8×10^{-5}	7.2×10^{-5}	2.1×10^{-6}	Dunlap, 1981
Air odor threshold: (ppm, v/v)		0.045		Amoore and Hautala, 1983

There are considerable differences in the reported vapor pressure data for p-nitrotoluene. For example, Verschueren (1983) lists the vapor pressure of p-nitrotoluene as 0.1 mm Hg at 20°C. Most of the values for vapor pressure at room temperature have been derived from extrapolation of data at higher temperatures. The vapor pressure data for p-nitrotoluene given by Lenchitz and Velicky (1970), however, was a measured value at room temperature. Amoore and Hautala (1983) have listed m-nitrotoluene in the odor safety class "B," which means ~50-90% of distracted persons perceive its presence at the TLV level (2 ppm, v/v).

The methyl group in the three nitrotoluenes can be chemically oxidized to the corresponding nitrobenzoic acid and the nitro-group can be chemically reduced to yield the corresponding toluidine. Nitration of the nitrotoluenes yields the corresponding dinitrotoluenes (Dunlap, 1981). In aqueous solution, o-nitrotoluene is oxidized with ozone and this oxidation is maximal at pH 6.5 (Munter et al., 1984)

1.3. PRODUCTION DATA

According to the TSCA Inventory (U.S. EPA, 1977), First Chemical Corp., Pascagoula, MI, and E.I. DuPont and Co., Deepwater, NJ, manufactured o-, m- and p-nitrotoluenes in the United States in 1977. The production volumes for the o-, m- and p-nitrotoluenes by the First Chemical Corp. were reported to range from 1-10 million pounds, 0.1-1 million pounds and 1-10 million pounds, respectively. The production volumes for the three isomers by the E.I. DuPont and Co. were not reported. In addition, Aceto Chemical Co., Inc., Flushing, NY, and DuPont and Co., Wilmington, DE, imported unspecified amounts of m-nitrotoluene in the United States in 1977. The only importer of p-nitrotoluene in the United States in 1977 was GAF Corp., New York, NY.

More recent production information about the three isomers of nitrotoluene (USITC, 1984; SRI, 1985) lists First Chemical Corp., and E.I. DuPont and Co. as manufacturers of the three isomers of nitrotoluene; however, no data on recent production volumes are available. The U.S. production volumes of o-nitrotoluene and p-nitrotoluene as of January, 1980, were estimated to be 28.7 and 15.4 million pounds, respectively (Dunlap, 1981).

Mononitrotoluenes are manufactured by nitration of toluene at controlled temperatures; the nitrating acid is a mixture of sulfuric and nitric acid. After completion of the reaction, the spent acid is withdrawn and the crude product is washed several times with dilute caustic soda and water. The product is then steam distilled to remove excess toluene, and the isomers are separated by a combination of fractional distillation and crystallization, yielding primarily o- and p-nitrotoluene (Dunlap, 1981).

1.4. USE DATA

o-Nitrotoluene is used in the manufacture of azo and sulfur-dyes, rubber chemicals and agricultural chemicals. p-Nitrotoluene is principally used in the production of intermediates for azo and sulfur dyes (Dunlap, 1981). m-Nitrotoluene is used in the preparation of toluidine, nitrobenzoic acid and dye intermediates (Windholz, 1983).

1.5. SUMMARY

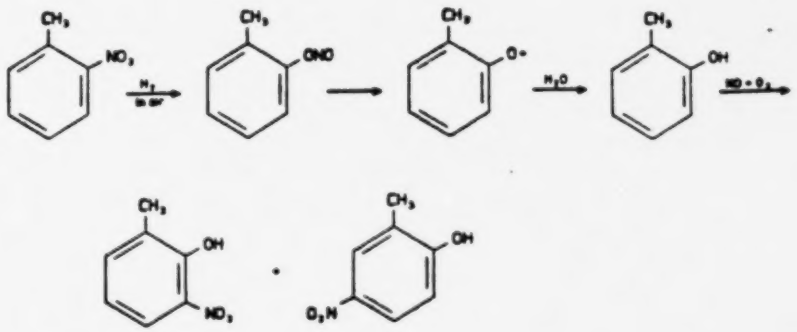
The mononitration of toluene results in the formation of three isomeric nitrotoluenes. o-Nitrotoluene and m-nitrotoluene are clear yellow liquids and p-nitrotoluene is a colorless solid at ambient temperature. The three isomers are slightly soluble in water but are readily soluble in organic solvents (Dunlap, 1981). The air odor threshold for m-nitrotoluene is such that 50-90% of distracted persons perceive warning of its presence at the TLV level of 2 ppm (Amoore and Hautala, 1983). In aqueous solution, o-nitrotoluene is oxidized with ozone at neutral pH (Munter et al., 1984).

Only two companies manufacture these chemicals in the United States, but the recent production volumes for these chemicals are not available. The total U.S. production volume of the three isomeric nitrotoluenes as of January, 1980, was estimated to be ~44 million pounds. The three nitrotoluenes are manufactured by nitration of toluene with a mixture of nitric acid and sulfuric acid, yielding primarily o- and p-nitroluene. The major use for the nitrotoluenes is in the manufacture of intermediates for azo and sulfur dyes (Dunlap, 1981).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. AIR

The sources of nitrotoluenes in the atmosphere are most likely their production and user facilities. The photooxidation of toluene in the atmosphere in the presence of NO_x produces m-nitrotoluene (Akimoto et al., 1978). Reports regarding the detection of nitrotoluenes in the atmosphere in the United States are rare; consequently, the fate of these compounds in the atmosphere has not been studied comprehensively. Nojima and Kanno (1977) studied the photochemical behavior of the three nitrotoluenes in the presence of NO_x in air by irradiating the air with a xenon lamp (wavelength >300 nm). The reaction was postulated to proceed in the following manner:



The percent disappearance in 5 hours of the reactants resulting from this reaction and the products formed with different nitrotoluenes are shown in Table 2-1. The authors failed to provide the reaction rates for these reactions. Therefore, it is impossible to estimate the half-life of this reaction under atmospheric conditions from the data provided by Nojima and Kanno (1977).

No kinetic data regarding the reaction of nitrotoluenes with hydroxyl radicals (OH•) were located in the available literature. The rate constant for nitrobenzene reaction with OH• radicals at 23°C is 0.21×10^{-12}

TABLE 2-1

Percent Disappearance and Products Identified from Irradiation of Nitrotoluenes in Air in the Presence of NO_x for 5 Hours*

Compound	Disappearance	Products Identified
o-Nitrotoluene	79%	2-methyl-6-nitrophenol 2-methyl-4-nitrophenol
m-Nitrotoluene	20%	3-methyl-2-nitrophenol 3-methyl-4-nitrophenol 3-methyl-6-nitrophenol
p-Nitrotoluene	38%	4-methyl-2-nitrophenol

*Source: Nojima and Kanno, 1977

cm^3 $molecule^{-1}$ sec^{-1} (Atkinson, 1985). If the concentration of OH• radicals in the atmosphere is assumed to be 10^6 molecules cm^{-3}, the estimated half-life for this reaction is ~40 days. Substitution of a methyl group in the benzene ring of nitrobenzene is expected to enhance its reactivity; the reaction rate of toluene with OH• radicals is ~5 times higher than the reaction rate of benzene (Atkinson, 1985). If a similar reactivity ratio is assumed for OH• reaction with nitrobenzene and nitrotoluene, the estimated half-life for nitrotoluene reaction would be ~8 days. Therefore, OH• reaction with atmospheric nitrotoluene may not be rapid.

Pertinent data regarding the transport of nitrotoluenes in the atmosphere were not located in the available literature. Since the vapor pressures of nitrotoluenes are $>10^{-7}$ mm Hg, vapor phase removal through adsorption onto particulate matter may not be a significant mechanism for atmospheric nitrotoluenes (Cupitt, 1980). If the nitrotoluenes are assumed to have physical removal mechanisms similar to those of nitrobenzene, significant removal of nitrotoluenes through wet and dry deposition seems unlikely (Cupitt, 1980).

2.2. WATER

Barltrop and Bunce (1968) irradiated a 1% solution of o-nitrotoluene in triethylamine with light of wavelength >290 nm from a medium pressure mercury arc lamp. Decomposition of the compound was 100% in 3 hours, and the products of decomposition were the corresponding amino-, azo-, 2-hydroxyazo- and azoxy-compounds. This reaction is probably insignificant in most natural waters since it proceeds with abstraction of hydrogen atoms from the solvent, which may not be possible in water. Zepp et al. (1984), however, showed that the humus-sensitized photoreaction of nitroaromatics in natural waters was significant. Using the intensity of midday sunlight and water from Aucilla River, FL, these authors estimated the first-order rate

constant for the sensitized photoreaction of o-nitrotoluene to be 0.92 $hour^{-1}$. Therefore, the estimated half-life for this reaction is ~45 hours, and it is likely to be a significant pathway for the removal of other nitrotoluenes from natural aquatic media as well.

The absolute rate constants for the reaction of m- and p-nitrotoluene with t-butylperoxy radicals at 30°C were reported to be 0.0105 and 0.0145 M^{-1} sec^{-1} (Howard and Chenier, 1973). If the rate constants for the peroxy radicals present in aquatic media are assumed to be similar to the above absolute rate constants and the concentration of peroxy radicals in natural aquatic media is assumed to be 10^{-9} M, it can be concluded from the estimated half-lives that this reaction is not likely to be a significant pathway for removal of nitrotoluene from aquatic media.

Of all the removal mechanisms, biodegradation of nitrotoluenes in aquatic media has been studied most frequently. The biodegradability of nitrotoluenes in aquatic media was studied with pure cultures of microorganisms, mixed microorganisms of natural origin and microorganisms obtained from sewage treatment conditions.

Villanueva (1960) reported that Nocardia v failed to use p-nitrotoluene as the sole source of carbon for growth from an aqueous medium containing 250 ppm of the substrate during a 16-day test. Nocardia v was chosen based on earlier reports regarding its catabolic versatility (Howard et al., 1976). Enzyme preparations from Verillonella alkalescens have been shown to transform nitrotoluenes to their corresponding amines in the presence of H_2 (McCormick et al., 1976). Among the nitrotoluenes, this reduction was fastest with p-nitrotoluene and slowest with o-nitrotoluene. A model wastewater purifier consisting of an aerator that was inoculated with Azotobacter agilis almost completely degraded ~100 ppm of o- and p-nitrotoluene (Bringmann and Kuehn, 1971).

The biodegradation of nitrotoluenes by mixed microorganisms of natural origin was studied by Alexander and Lustigman (1966). When solutions of o-, m- and p-nitrotoluenes of original concentrations of ~10 ppm were incubated with a 1% suspension of Niagara silt loam, the parent compounds degraded completely in >64 days. In other words, biodegradation for the nitrotoluenes by the microorganisms used in this study was insignificant.

The reported observations about the biodegradability of nitrotoluenes under normal sewage treatment conditions are conflicting. In the Japanese MITI test, where 100 ppm substrate was incubated with 30 ppm unacclimatized activated sludges at 25°C for 2 weeks, all three nitrotoluenes were found to be degradation resistant (Kawasaki, 1980; Kitano, 1978). On the other hand, Thom and Agg (1975) concluded from their experimental results that the three nitrotoluenes should be biodegradable by biological sewage treatment provided suitable acclimatization of the microorganisms can be achieved. A similar conclusion was reached by Pitter (1976), who used adapted activated sludge as inoculum for studying the biodegradation of 125 ppm nitrotoluene isomers. He observed ~98% removal of the compounds after 5 days of incubation. Hallas and Alexander (1983) measured the transformation of m- and p-nitrotoluene in sewage effluents amended with 10 ppm of substrate under both aerobic and anaerobic conditions. Both m- and p-nitrotoluene disappeared completely within 14 days under anaerobic conditions, and toluidine was detected as one of the primary products. Although p-nitrotoluene degraded completely in 14 days under aerobic conditions, the degradation of m-nitrotoluene was not complete. From the degradation curve given by the authors, the estimated half-lives for m- and p-nitroluenes were 10 and 20 days, respectively, under aerobic conditions and 8 and 5 days, respectively, under anaerobic conditions.

Pertinent data regarding the transport processes of nitrotoluenes in aquatic media could not be located in the available literature as cited in the Appendix. From their physical characteristics, it is anticipated that nitrobenzene, 2,4-dinitrotoluene and 2,6-dinitrotoluene will not significantly volatilize from most aquatic media (Callahan et al., 1979). Therefore, it is also likely that nitrotoluenes will not volatilize significantly from aquatic media. The same conclusions can be reached from the values of estimated Henry's law constants (see Section 1.2.) and the expected volatility of the compounds as given by Lyman et al. (1982). The potential for the adsorption of nitrotoluenes by suspended particulate matter and sediments in aquatic media can be predicted from its K_{oc}. The K_{oc} values for the three nitrotoluenes can be estimated from their K_{ow} values (see Section 1.2.) by using the following equation: $\log K_{oc} = 0.544 \log K_{ow} + 1.377$ (Lyman et al., 1982). Given the estimated log K_{oc} values of 2.63, 2.76 and 2.67 for o-, m- and p-nitrotoluenes, respectively, it can be predicted that some sorption of these compounds onto sediments and suspended particulate matter is likely to occur in aquatic media.

The overall half-lives of o-, m- and p-nitrotoluene in river water as a result of a combination of biological, chemical and physical degradation processes have been estimated to be 3.2, 2.7 and 2.7 days, respectively (Zoeteman et al., 1980).

Experimentally determined BCFs for the three isomers of nitrotoluenes in aquatic organisms were not available; however, from the Japanese MITI tests, Sasaki (1978) concluded that both o- and p-nitrotoluenes do not bioaccumulate significantly in aquatic organisms. The BCF can be estimated from the K_{ow} values by using the following equation: $\log BCF = 0.85 \log K_{ow} - 0.70$ (Lyman et al., 1982). The estimated log BCF values by this method are 1.26,

1.38 and 1.31 for o-, m- and p-nitrotoluene. Therefore, these chemicals are not expected to significantly bioaccumulate in aquatic organisms.

2.3. SOIL

The fate of nitrotoluenes in soil is not well studied. The fate of nitrotoluenes as a result of tank filtration and dune filtration was determined by Zoeteman et al. (1980), Piet et al. (1981) and Piet and Morra (1983). All of these studies demonstrate that nitrotoluenes would completely degrade in the top 50 cm of the soil. It was also demonstrated that the removal rate depends on the initial concentrations of the nitrotoluenes and that removal may not be complete at higher (unspecified) concentrations (Piet et al., 1981). It can also be speculated from these studies that at low (µg/ℓ) concentrations, nitrotoluene may not leach from soil to groundwater. Considering the low volatility of nitrotoluenes from aquatic media (see Section 2.2.), it is unlikely that significant evaporation will occur from soil.

2.4. SUMMARY

The atmospheric fate of nitrotoluenes has not been studied comprehensively. Because of their reaction with NO_x and sunlight, it is likely that significant removal of nitrotoluenes from the atmosphere will occur (Nojima and Kanno, 1977); however, the half-life for this reaction cannot be estimated because kinetic data was not available. Significant removal of nitrotoluenes from the atmosphere through physical removal processes such as wet and dry deposition is unlikely. The data base for nitrotoluenes is inadequate for accurate prediction of their atmospheric fates and lifetimes.

Humus-sensitized photoreaction of nitrotoluenes may remove significant amounts of these compounds from natural waters; the half-life for this reaction is estimated to be 3 days (Zepp et al., 1984). The removal of nitrotoluenes from most natural waters through biodegradation may not be significant (Alexander and Lustigman, 1966). Although the Japanese MITI test lists these compounds as degradation resistant (Kawasaki, 1980; Kitano, 1978), significant biodegradation of nitrotoluenes will occur under sewage treatment conditions with acclimatized activated sludge (Pitter, 1976; Hallas and Alexander, 1983). The significant removal of nitrotoluenes from aquatic media through volatilization is not likely, but removal of some nitrotoluenes from the aquatic phase by sorption onto suspended particulate matter may occur. The overall half-life of the three nitrotoluenes in river water is estimated to be ~3 days (Zoeteman et al., 1980). Significant bioconcentration of these compounds in aquatic organisms is unlikely (Sasaki, 1978).

Data pertaining to the fate of nitrotoluenes in soil are inadequate. At lower concentrations, nitrotoluenes are not likely to leach from soil to groundwater (Zoeteman et al., 1980; Piet et al., 1981; Piet and Morra, 1983). It has been speculated that a significant loss of nitrotoluenes through volatilization from soil will not occur.

3. EXPOSURE

3.1. AIR

The only atmospheric monitoring data available for nitrotoluenes in the United States were reported by Pellizzari (1978). The ambient air levels of o- and p-nitrotoluene were measured at 12 sampling points surrounding the E.I. DuPont and Co., Deepwater, NJ, facility in June, 1977. At 10/12 sampling points, no nitrotoluene was detected, while at two other sampling points, o-nitrotoluene was detected at trace levels and 47 ng/m^3. The concentrations of p-nitrotoluene at the two sampling points where this compound was detected were 59 and 86 ng/m^3.

3.2. WATER

Nitrotoluenes have been detected in wastewater from TNT production facilities, chemical plants and paper mills. o-Nitrotoluene was detected at an unspecified concentration in the wastewater of a treatment lagoon from a paper mill (Howard et al., 1976). p-Nitrotoluene was reported at a concentration of 0.04 ppm in the waste treatment lagoon of a chemical manufacturing plant (Howard et al., 1976). The concentration of o-nitrotoluene in wastewater from TNT manufacturing facilities was reported to range between 0.15 and 16 ppm. The corresponding concentrations of p-nitrotoluene ranged between 0.12 and 9.2 ppm (Howard et al., 1976). With modern treatment processes, however, the wastewaters may contain smaller amounts of nitrotoluene. Pearson et al. (1979) and Spanggord and Suta (1982) detected both o- and p-nitrotoluene in at least 10% of the wastewater samples from TNT production facilities. The 90th percentile concentrations of o- and p-nitrotoluene were 0.030 and 0.100 ppm, respectively, in these samples.

Nitrotoluenes have also been detected in surface waters. DeWalle and Chian (1978) detected nitrotoluene (isomers not specified) at a frequency of 10% in Delaware River water near the Philadelphia-Wilmington area. The average quarterly concentrations of o- and p-nitrotoluene (not separated) in the River Waal, the Netherlands, ranged between 3.9 and 5.8 ppb in 1974, with a maximum concentration of 18.1 ppb. The average quarterly concentrations of o- and p-nitrotoluene (not separated) in the River Maas ranged between none detected and 0.2 ppb, with a maximum value of 0.3 ppb (Meijers and Van der Leer, 1976). The concentrations of o-, m- and p-nitrotoluene in the River Lobith, the Netherlands, were reported to be 3.0, 0.3 and 1.0 ppb, respectively (Zoeteman et al., 1980). The concentrations of o-, m- and p-nitrotoluene in the Rhine River in the Netherlands were reported to be 10, 1 and 10 ppb, respectively (Piet and Morra, 1983). The U.S. EPA (1985) reported that o-nitrotoluene was detected at a concentration of 280 ppm in one sediment sample from the Niagara River, NY. None of the isomers of nitrotoluene have ever been detected in drinking water either in the United States or elsewhere (Howard et al., 1982).

3.3. FOOD

Pertinent data regarding the detection of nitrotoluenes in foods were not located in the available literature cited in the Appendix.

3.4. SUMMARY

The only available atmospheric monitoring data in the United States reported the presence of o- and p-nitrotoluene at concentrations ranging from trace levels to 86 ng/m^3 in 2/12 samples collected around a manufacturing facility (Pellizzari, 1978). Nitrotoluenes have been detected in wastewater from paper mills, TNT production facilities and chemical plants.

The concentrations of o- and p-nitrotoluene in wastewater from TNT production facilities may be as high as 16 and 9.2 ppm, respectively (Howard et al., 1976). With recent treatment technologies, however, the level of o- and p-nitrotoluene in TNT wastewater samples at the 90th percentile concentration may be 0.030 and 0.100 ppm, respectively (Pearson et al., 1979). Nitrotoluenes have been detected in several surface waters. In Rhine River water in the Netherlands, the concentrations of o-, m- and p-nitrotoluenes have been reported to be 10, 1 and 10 ppb, respectively (Piet and Morra, 1983). Nitrotoluenes have not been detected in drinking water or in foods either in the United States or elsewhere in the world.

4. PHARMACOKINETICS

4.1. ABSORPTION

Chism et al. (1984) treated male Fischer 344 rats by gavage with a single dose (200 mg/kg) of o-, m- or p-[ring-U-^{14}C]-nitrotoluene in corn oil and studied the excretion of radioactivity. At 72 hours after dosing, 85.8, 67.8 and 76.7% of the administered radioactivity from the o-, m- and p-isomers, respectively, were detected in the urine. The majority of the radioactivity (73-86%) was excreted in urine, feces and expired air in the first 24 hours, indicating that the nitrotoluenes were absorbed rapidly and extensively from the gastrointestinal tract of rats. By 72 hours after treatment, 80-90% of the administered dose was recovered in the urine, feces and expired air.

Absorption following inhalation and dermal exposure can be inferred from the demonstrated toxicity following occupational exposure (Chapter 5). Although dermal absorption has been observed in the rat (NIOSH, 1985), Kinkead et al. (1977) reported no signs of toxicity in rabbits exposed dermally to o-, m- or p-nitrotoluene.

4.2. DISTRIBUTION

Pertinent data regarding the distribution of the nitrotoluenes could not be located in the available literature as cited in the Appendix. However, based on the demonstrated target organ toxicity (lung, liver, kidney, hematopoietic system and reproductive system) in rodents (Chapter 5), the nitrotoluenes appear to be well distributed.

4.3. METABOLISM

As reviewed by Williams (1959), the metabolism of o- and p-nitrotoluenes was first studied by Jaffe (1874, 1878-1879) in dogs. o-Nitrotoluene was

oxidized at the methyl group to o-nitrobenzyl alcohol, which was excreted as a glucuronide (25% of the dose), and o-nitrobenzoic acid (10% of the dose). p-Nitroluene was metabolized to p-nitrobenzoic and p-nitrohippuric acid.

Metabolism of nitrotoluenes to nitrobenzoic acids appears to be a common pathway among mammalian species. Chakraborty and Smith (1967) found that incubation of the postmitochondrial supernatant fraction from liver homogenates of rabbits, coypus, hamsters, guinea pigs, cats or rats with p-nitrotoluene resulted in the formation of p-nitrobenzoic acid.

Gillette (1959) found that p-nitrotoluene was oxidized to p-nitrobenzyl alcohol in rabbit liver microsomal preparations; the oxidization was NADPH-dependent. Subsequent oxidation to p-nitrobenzoic acid was catalyzed by liver cytosolic preparations. Oxidation of o-, m- and p-nitrotoluene to the respective nitrobenzyl alcohols by rat liver microsomes was NADPH-dependent and inhibited by carbon monoxide, indicating that the first step in the metabolism of the nitrotoluenes is cytochrome P-450-catalyzed oxidation to nitrobenzyl alcohol (de Bethizy and Rickert, 1984).

de Bethizy and Rickert (1984) compared the <u>in vitro</u> metabolism of o-, m- and p-nitrotoluene using isolated hepatocytes or hepatic microsomes from Fischer 344 rats. Quantitative and qualitative differences were observed among the metabolites formed when the three isomers were incubated individually for 45 minutes with hepatocytes (Table 4-1). When the individual isomers were incubated with rat liver microsomes, only the nitrobenzyl alcohols were formed, indicating that microsomal metabolism did not account for the qualitative differences in the metabolites. Only small differences were observed in the rates of microsomal oxidation of the three isomers, suggesting that the quantitative differences could not be explained by differences in the rate of formation of nitrobenzyl alcohol.

TABLE 4-1

Qualitative and Quantitative Comparison of Metabolites Formed Following Incubation of o-, m- or p-Nitrotoluene with Rat Hepatocytes[a]

Metabolite[b]	% Total Metabolism ± SD		
	o-Nitrotoluene	m-Nitrotoluene	p-Nitrotoluene
Nitrobenzyl alcohol	52.4±4.6	29.4±3.3	12.4±2.9
Nitrobenzyl glucuronide	27.6±5.1	13.1±1.9	2.0±0.3
Nitrobenzyl sulfate	ND	ND	3.9±1.2
S-(Nitrobenzyl)glutathione	ND	ND	67.7±9.0
Nitrobenzoic acid	2.9±0.6	55.9±5.3	2.1±0.3

[a]Source: de Bethizy and Rickert, 1984

[b]The metabolites are the o-, m- or p-isomers corresponding to the respective isomers of nitrotoluene

ND = Not detected

Besides differences in metabolism, differences in the toxicity of the nitrotoluenes have been observed (Chapter 5). Investigations of the differential toxicity have led to elucidation of subsequent metabolic processes. Covalent binding of radioactivity to hepatic macromolecules, particularly to DNA, was much greater following dosing of rats with o-[^{14}C]-nitrotoluene than with m- or p-[^{14}C]-nitrotoluene (Rickert et al., 1984). Pretreatment of the rats with sulfotransferase inhibitors inhibited the covalent binding of radioactivity from o-[^{14}C]-nitrotoluene to liver macromolecules in general by ~60% and to DNA by >96%. Sulfotransferase inhibitors did not affect the binding of radioactivity from m- or p-[^{14}C]-nitrotoluene. The results indicated that sulfation played an important role in the activation of o-nitrotoluene to the toxic species, although no sulfur-containing metabolites of o-nitrotoluene (or m-nitrotoluene) were formed *in vitro* by rat hepatocytes.

Radioactivity from the [^{14}C]-nitrotoluene isomers administered orally to rats appeared in the intestines and portal blood 12 hours before peak covalent binding of radioactivity (which occurred at 12 hours) to hepatic macromolecules (Long and Rickert, 1983). These results suggested the involvement of enterohepatic recirculation in the generation of the reactive species; however, studies directly measuring biliary excretion (i.e., using bile duct-cannulated rats) were not located in the available literature.

Doolittle et al. (1983) demonstrated the importance of metabolism by intestinal microflora on the expression of genotoxicity of o-nitrotoluene. When o-, m- or p-nitrotoluene were tested *in vitro* for UDS in isolated rat hepatocytes, negative results were obtained. *In vivo*, only o-nitrotoluene induced UDS by hepatocytes from male rats that had been treated orally with the isomers. When hepatocytes from germ-free male rats treated with o-nitrotoluene were tested, no UDS was detected; however, UDS was induced in

o-nitrotoluene-treated germ-free male rats that had been inoculated with Charles River Altered Schaedler Flora. Therefore, activation of o-nitrotoluene probably occurs by metabolism by bacteria in the intestine rather than by enzymes in the liver. All tests using female rats were negative. Qualitative and quantitative analysis of intestinal microflora revealed no significant differences between the sexes; therefore, the sex difference in response to o-nitrotoluene may be due to differences in hepatic metabolism or disposition or both. Doolittle et al. (1983) and Long and Rickert (1983) suggested that differences in biliary excretion of glucuronides may account for differences in the toxicities of the isomers as well as for the different responses of male and female rats to o-nitrotoluene.

Chism et al. (1984) compared the in vivo metabolism of o-, m- and p-(ring-U-^{14}C]-nitrotoluene in male Fischer 344 rats treated by gavage with 200 mg/kg of the respective isomers. Urine was collected for 72 hours and analyzed by HPLC and GC/MS for metabolites. As seen from Table 4-2, quantitative and qualitative differences were found among the three isomers. In addition to the metabolites listed in Table 4-2, two unknown metabolites of o-nitrotoluene were detected and amounted to ~16 and 6% of the dose. These metabolites were suspected to be sulfur-containing conjugates of 2-acetamidotoluene and 2-aminotoluene, respectively, but the identities were not confirmed. Two minor unidentified metabolites of m-nitrotoluene, amounting to 2.2 and 2.6% of the dose, were also detected. Based upon these results, and in vitro studies of de Bethizy and Rickert (1983, 1984), Chism et al. (1984) proposed the metabolic pathway illustrated in Figure 4-1. According to the scheme, metabolism of all three isomers proceeds by oxidation of the methyl group to nitrobenzyl alcohol, which undergoes further metabolism by three main pathways. The first pathway (I) involves oxidation of nitrobenzyl alcohol to nitrobenzoic acid. Nitrobenzoic acid was a major

TABLE 4-2

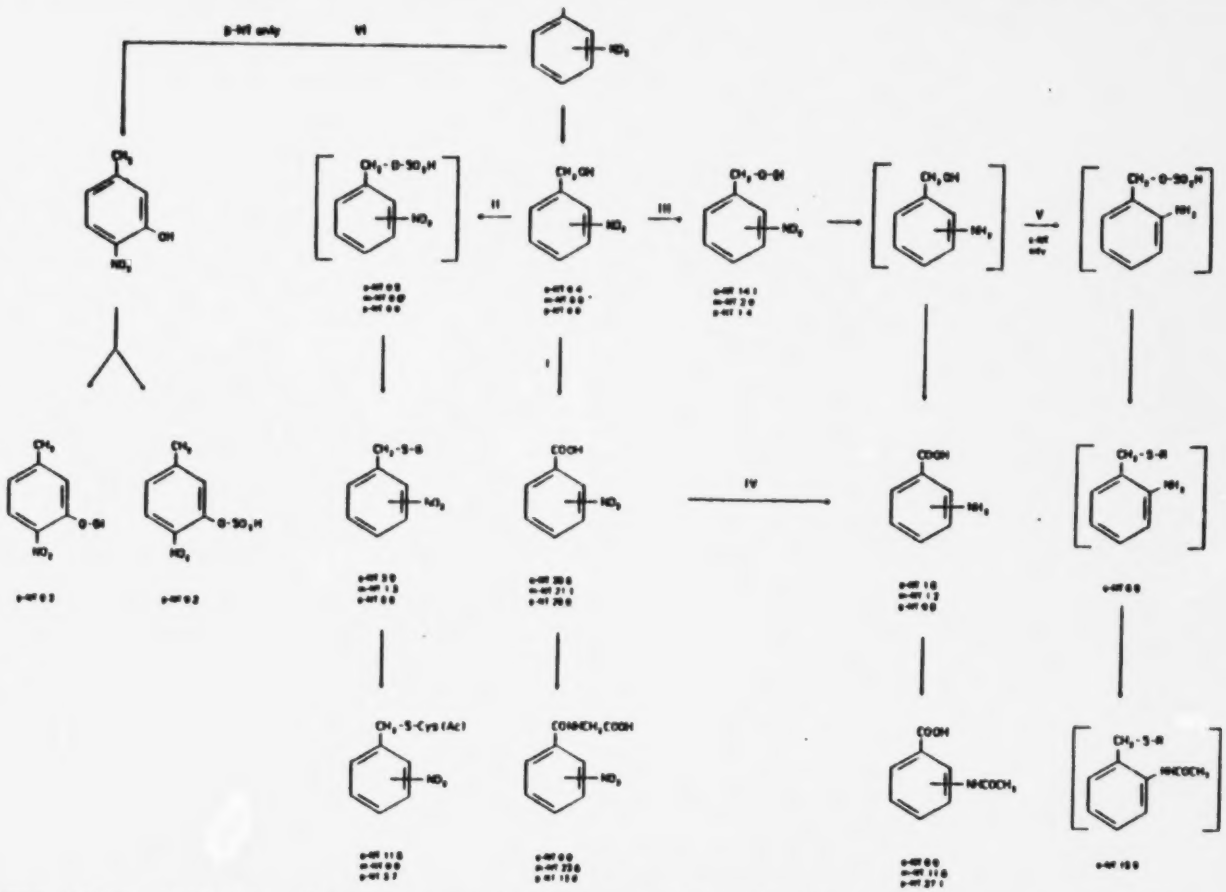
Qualitative and Quantitative Comparison of Metabolites Found in the Urine of Rats 72 Hours After Oral Dosing With o-, m- or p-Nitrotoluene[a]

Metabolite[b]	% of Dose ± SEM		
	o-Nitrotoluene	m-Nitrotoluene	p-Nitrotoluene
Nitrobenzoic acid	28.6±0.3	21.1±1.1	28.0±2.6
Nitrobenzyl glucuronide	14.1±0.7	2.0±0.1	1.4±0.1
S-(Nitrobenzyl)-N-acetylcysteine	11.6±0.2	0	3.7±0.1
S-(Nitrobenzyl) glutathione	3.9±0.4	1.3±0.1	0
Aminobenzoic acid	1.8±0.2	1.2±0.3	0.8±0.1
Nitrobenzyl sulfate	0.5±0.2	0	0
Nitrobenzyl alcohol	0.4±0.3	0	0
Acetoamidobenzoic acid	0	11.6±0.4	27.1±3.0
Nitrohippuric acid	0	23.6±2.0	13.0±0.7
5-Methyl-2-nitrophenyl glucuronide	0	0	0.3±0.0
5-Methyl-2-nitrophenyl sulfate	0	0	0.2±0.0

[a]Source: Chism et al., 1984

[b]The metabolites are the o-, m- or p-isomers corresponding to the isomers of nitrotoluene administered.

SEM = Standard error of the mean



The values below each structure represent the percentage of the dose of each metabolite excreted in the urine 72 hours after a 200 mg/kg oral dose of o-, m- or p-nitrotoluene. Structures in brackets are postulated intermediates or inconclusively identified metabolites (see text). S-G = glutathione; S-Cys(Ac) = N-acetylcysteine; Gl = glucuronide; S-R = sulfur-containing conjugate.

FIGURE 4-1

Proposed Pathway of Metabolism of Nitrotoluene (NT) in Male Fischer Rats

Source: Chism et al., 1984

urinary metabolite of all three isomers; however, oxidation of nitrobenzyl alcohol to nitrobenzoic acid was a major route only for o-nitrotoluene in vitro (de Bethizy and Rickert, 1984). In pathway I, m- and p-nitrobenzoic acids are metabolized further to the corresponding glycine conjugates, m- and p-nitrohippuric acids; the nitrohippuric acids were major urinary metabolites of m- and p-nitrotoluene, but not o-nitrotoluene (Chism et al., 1984). As discussed by Williams (1959), o-nitrobenzoic acid itself does not form a conjugate with glycine.

In the second pathway (II), conjugation of o-, m- or p-nitrobenzyl alcohol with glutathione, through the intermediacy of a nitrobenzyl sulfate (de Bethizy and Rickert, 1983), results in the corresponding S-(nitrobenzyl) glutathione conjugates; glutathione conjugates were excreted in the urine of rats treated with o- or m-nitrotoluene, but not p-nitrotoluene (Chism et al., 1984). Only the metabolism of p-nitrotoluene resulted in sulfur-containing metabolites in vitro (de Bethizy and Rickert, 1983, 1984). The glutathione conjugates of the o- and p-isomers, but not the m-isomers, were further metabolized to o- and p-S-(nitrobenzyl)-N-acetylcysteine.

In the third pathway (III), which appears to be the major pathway for in vivo metabolism of o-nitrotoluene, conjugation of o-, m- or p-nitrobenzyl alcohol with glucuronic acid in rats may result in the formation of aminoand acetamidobenzoic acids as follows: nitrobenzyl glucuronide may be deconjugated during biliary excretion and the nitro groups reduced by intestinal microflora, resulting in aminobenzyl alcohols, which may undergo enterohepatic recirculation. Further oxidation and acetylation in the liver would result in aminobenzoic acid and acetamidobenzoic acid. Alternately, in the fourth pathway (IV), nitrobenzoic acid may be actively transported from the systemic circulation of rats into the intestine, where microflora reduce nitrobenzoic acid to aminobenzoic and acetamidobenzoic acids (Gardner and

Renwick, 1978). Although metabolism of nitrotoluenes to aminobenzoic acids may depend upon reduction of nitrobenzoic acid by intestinal microflora in rats, other species may reduce nitrobenzoic acid directly in the liver. Fouts and Brodie (1957) found that liver slices from mice or guinea pigs were ~3 times more active in reducing p-nitrobenzoic acid to p-aminobenzoic acid than were liver slices from rabbits; liver slices from dogs or rats had relatively little activity.

The unknown metabolites of o-nitrotoluene that were tentatively identified as sulfur-containing conjugates of o-acetamidotoluene and o-aminotoluene could arise from a benzyl sulfate intermediate formed from aminobenzyl alcohol (V) (Chism et al., 1984).

Finally, pathway VI was proposed to account for the appearance of small amounts of 5-methyl-2-nitrophenyl glucuronide and 5-methyl-2-nitrophenyl sulfate in the urine of rats treated with p-nitrotoluene (Chism et al., 1984).

4.4. EXCRETION

Rats treated by gavage with o-, m- or p-[ring-U-^{14}C]-nitrotoluene eliminated ~80-90% of the radioactivity in the urine, feces and expired air within 72 hours of dosing (Table 4-3) (Chism et al., 1984). The urine was the major route for all three isomers. Much of the fecal excretion may have resulted from biliary excretion. The expired air appeared to be a minor route of elimination for o- and m-nitrotoluene, but not for p-nitrotoluene. Most of the radioactivity (73-86% of the dose) was excreted by all routes within the first 24 hours. Peak excretion rates occurred within 3-10 hours for urine, 24 hours for feces and 12 hours for expired air. The identity and amounts of urinary metabolites of the three nitrotoluenes are presented in Table 4-2.

TABLE 4-3

Excretion of Radioactivity by Male Fischer 344 Rats 72 Hours After a Single Oral Dose (200 mg/kg) of o-, m- or p-[^{14}C]-Nitrotoluene[a]

Route of Excretion	% of Dose Excreted[b]		
	o-Nitrotoluene	m-Nitrotoluene	p-Nitrotoluene
Urine	85.8±1.7	67.8±2.3	76.7±2.6
Feces	4.6±1.3	12.5±2.3	6.1±0.7
Expired air	0.1±0.0	0.1±0.0	0.0±0.0

[a]Source: Chism et al., 1984

[b]Values represent the mean ±SEM for three rats.

4.5. SUMMARY

The nitrotoluenes were absorbed extensively from the gastrointestinal tract of rats following oral dosing (Chism et al., 1984). Absorption following inhalation and dermal exposure can be inferred from the demonstrated toxicity following occupational exposure (Chapter 5). Information regarding the distribution of nitrotoluenes was not available. The data on metabolism indicate that following absorption by the gastrointestinal tract, the nitrotoluenes are oxidized to nitrobenzyl alcohols by microsomal cytochrome P-450 in the liver (Gillette, 1959; de Bethizy and Rickert, 1984). Subsequent oxidation to nitrobenzoic acids occurs in the cytosolic fraction of hepatocytes (de Bethizy and Rickert, 1984). Metabolism to nitrobenzoic acids appears to be a common process among mammalian species (Chakraborty and Smith, 1967). In rats, conjugation of nitrobenzoic acids with glycine results in the formation of nitrohippuric acids (Chism et al., 1984). Nitrobenzyl alcohols can also be sulfated and conjugated with glutathione or conjugated with glucuronic acid. Glucuronides are excreted in the bile (or nitrobenzoic acid may be secreted) into the intestine, where they are reduced by intestinal microflora (Long and Rickert, 1983; Doolittle et al., 1983; Chism et al., 1984). The reduced metabolites can be reabsorbed by enterohepatic recirculation into the liver, where a reactive species binds to hepatic macromolecules and damages DNA (Doolittle et al., 1983; Long and Rickert, 1983; Rickert et al., 1984). Quantitative and qualitative differences in these processes for the three isomers in rats result in quantitative and qualitative differences in <u>in vitro</u> metabolites, urinary metabolites and the expression of toxic effects (de Bethizy and Rickert, 1984; Chism et al., 1984). Between 80 and 90% of orally administered radioactivity from [^{14}C]-nitrotoluenes was excreted in the urine, feces and expired air within 72 hours; the urinary route was the major route (Chism et al., 1984).

5. EFFECTS

5.1. CARCINOGENICITY

Testing by the National Toxicology Program of p-nitrotoluene for carcinogenicity by gavage treatment of rats and mice is in progress; the preliminary subchronic study has been completed (NTP, 1985). The o- and m-isomers are not scheduled for testing.

Slaga et al. (1985) tested o- and p-nitrotoluenes (98% pure) as tumor initiators on the skin of mice. An unspecified number of SENCAR mice were treated topically with single applications of 24, 120 or 240 mg of o-nitrotoluene in acetone or 50, 250 or 400 mg of p-nitrotoluene in acetone. Treatment with the nitrotoluenes was followed by weekly applications of 4 µg TPA for 30 weeks. p-Nitrotoluene was negative as a tumor initiator in this study. Initiation with 240 mg o-nitrotoluene followed by promotion with TPA resulted in skin carcinomas in 5% and papillomas in 16% of the mice compared with 2.5 and 11%, respectively, of mice treated with TPA alone; however, the differences were not statistically significant. Furthermore, comparisons of papillomas/mouse revealed no statistically significant difference. It was concluded that o-nitrotoluene might be a weak skin tumor initiator.

Slaga et al. (1985) also tested o- and p-nitrotoluenes in a lung tumor assay in strain A/Jax male mice. Groups of 30 mice were injected intraperitoneally with o-nitrotoluene in corn oil at 840, 2100 or 4800 mg/kg or p-nitrotoluene in corn oil at 1800, 4500 or 9000 mg/kg, 3 days/week for 8 weeks. The mice were killed 16 weeks after the last injection. Doses were selected from preliminary toxicity studies where the MTD was determined for each isomer. The MTD was defined as the highest dose that resulted in no mortality when injected 3 times/week for 2 consecutive weeks. Although the

percentage of survivors with lung tumors and the number of tumors/lung increased in an apparently dose-related manner for both isomers, no statistically significant differences were found between nitrotoluene-treated mice, corn oil-treated controls or untreated controls. It was concluded that the nitrotoluenes did not result in "an unequivocally positive response." Therefore, data are insufficient to conclude that the m- and p-nitrotoluenes are carcinogenic, but suggest that the o-isomer may be a weak skin tumor initiator.

5.2. MUTAGENICITY

The nitrotoluenes have been tested for mutagenicity and genotoxicity with different responses depending upon the isomer tested (Table 5-1). m-Nitrotoluene was negative for reverse mutation in all strains of S. typhimurium tested with and without S-9 (Haworth et al., 1983; Miyata et al., 1981; Suzuki et al., 1983; Tokiwa et al., 1981; Spanggord et al., 1982) and in the presence of the co-mutagen, norharman (Suzuki et al., 1983). m-Nitrotoluene was also negative for UDS in rat hepatocytes when tested in vitro or in vivo (Butterworth et al., 1982; Doolittle et al., 1983), and for chromosome aberrations in Chinese hamster ovary cells; however, a weakly positive response was obtained for SCE in Chinese hamster ovary cells (Galloway et al., 1983).

Results of reverse mutation testing with p-nitrotoluene were equivocal. Positive results for reverse mutation were obtained in S. typhimurium TA100 with and without S-9 by Miyata et al. (1981), Spanggord et al. (1982) and Melnikow et al. (1981). Negative results, however, were obtained in several strains of S. typhimurium, including TA100, with and without S-9 by Haworth et al. (1983), Suzuki et al. (1983), Tokiwa et al. (1981) and Chiu et al. (1978). p-Nitrotoluene was also negative in TA98 in the presence of S-9 and

TABLE 5-1

Mutagenicity Testing of Nitrotoluenes

Assay	Indicator/ Organism	Isomer	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA100, TA1535, TA1537, TA98	o-, m- or p-	plate incorporation	3.3-10,000 µg/plate	±S-9	-	All three isomers were negative in all strains with and without S-9 prepared from Aroclor induced rats or hamsters.	Haworth et al., 1983
Reverse mutation	S. typhimurium TA92 TA1535 TA100 TA1537 TA94 TA98	o-, m- or p-	plate incorporation	30-3000 µg/plate	±S-9	- - ± (p) - - -	Slight but significant increase in the number of revertants in TA100 at 100-1000 µg/plate of p-nitrotoluene in the presence of S-9.	Miyata et al., 1981
Reverse mutation	S. typhimurium TA98, TA100	o-, m- or p-	plate incorporation	0-300 or 100-1000 µg/plate	±S-9	-	All isomers were negative in both strains with and without S-9.	Suzuki et al., 1983; Tokiwa et al., 1981
Reverse mutation	S. typhimurium TA98	o-, m- or p-	plate incorporation	100-1000 µg/plate	+S-9, + norharman +S-9, - norharman	± -	Only the o-isomer was positive with S-9 and norharman.	Suzuki et al., 1983
Reverse mutation	S. typhimurium TA1535, TA1537 TA1538, TA98, TA100	o- m- p-	plate incorporation	10-5000 µg/plate	±S-9	- - ‡	The p-isomer was positive with and without S-9 in TA100.	Spanggord et al., 1982
Reverse mutation	S. typhimurium TA98, TA100	o- or p-	plate incorporation	0.1, 1 or 10 µmol/plate	none	-	Both isomers were negative in both strains. No S-9 was used because the TA98 and TA100 strains used contained nitroreductase.	Chiu et al., 1978
Reverse mutation	S. typhimurium TA98, TA100	p-	plate incorporation	NR	-S-9	+	The isomer was tested with and without S-9, and the response was positive without S-9; however, it was unclear if the response was positive in both strains and if the response was positive in the presence of S-9.	Melnikow et al., 1981

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TABLE 5-1 (cont.)

Assay	Indicator/ Organism	Isomer	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Mitotic gene conversion	Saccharomyces cerevisiae	p-	NR	NR	NR	+	The abstract provided no further details.	Marquardt et al., 1970
UDS	F344 rat hepatocytes	o- m- p-	cell incubation	10-1000 µM	none	- - -	All negative.	Butterworth et al., 1982; Doolittle et al., 1983
UDS	male F344 rat hepatocyte	o- m- p-	gavage	200 or 500 mg/kg	none	+ - -	Only the o-isomer was positive in vivo in male rats; all isomers were negative in female rats.	Butterworth et al., 1982; Doolittle et al., 1983
UDS	germ-free male rat hepatocytes	o-	gavage	200 or 500 mg/kg	none	-	NC	Butterworth et al., 1982; Doolittle et al., 1983
UDS	hepatocytes from germ-free male rats inoculated with exogenous microflora	o-	gavage	200 or 500 mg/kg	exogenous microflora	+	Intestinal microflora were necessary for o-nitrotoluene to elicit a positive response	Butterworth et al., 1982; Doolittle et al., 1983
Chromosome aberrations	Chinese hamster ovary cells	o- m- p-	cell culture	NR	±S-9	= = +	It was not stated if p-nitrotoluene was positive with and or without S-9	Galloway et al., 1983
SCE	Chinese hamster ovary cells	o- m- p-	cell culture	NR	±S-9	= + (weak) +	It was not stated if m- and p-nitrotoluene were positive with and/or without S-9	Galloway et al., 1983

NR = Not reported; NC = no comment

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norharman (Suzuki et al., 1983). p-Nitrotoluene was positive for mitotic gene conversion in S. cerevisiae (Marquardt et al., 1970) and for SCE and chromosome aberrations in Chinese hamster ovary cells (Galloway et al., 1983). It was not clear if metabolic activation was required in these tests.

o-Nitrotoluene was negative with and without S-9 for reverse mutation in all strains of S. typhimurium tested (Haworth et al., 1983; Miyata et al., 1981; Suzuki et al., 1983; Tokiwa et al., 1981; Spanggord et al., 1982; Chiu et al., 1978) and for SCE and chromosome aberrations in Chinese hamster ovary cells (Galloway et al., 1983). o-Nitrotoluene was negative for UDS in rat hepatocytes in vitro (see Section 4.3), but was positive for UDS when administered orally to male rats (Butterworth et al., 1982; Doolittle et al., 1983). When o-nitrotoluene was administered to germ-free male rats, a negative response for UDS was obtained; however, when the germ-free rats were inoculated with Charles River Altered Schaedler Flora in the intestines, o-nitrotoluene gave a positive response for UDS in hepatocytes. Therefore, intestinal microflora played a role in the bioactivation of o-nitrotoluene to a metabolite capable of damaging liver DNA.

5.3. TERATOGENICITY

In a 6-month oral study, groups of 10 male and 10 female Wistar rats (200 g) were treated by gavage 5 days/week for 6 months with o-nitrotoluene in olive oil at 0 or 200 mg/kg/day, with m-nitrotoluene in olive oil at 0 or 300 mg/kg/day or with p-nitrotoluene in methyl cellulose at 0 or 400 mg/kg/day (Ciss et al., 1980). The doses were determined from a preliminary range finding study which was not described in the report. After the first 3 months of treatment, the following matings for each isomer were performed: five control males with five control females, five control males with five treated females, five treated males with five control females and five

treated males with five treated females. Treatment was continued during gestation. The dams were allowed to deliver at term. The only effects on the offspring were slight histological lesions in the spleens of pups from parents treated with m-nitrotoluene.

5.4. OTHER REPRODUCTIVE EFFECTS

In the study by Ciss et al. (1980) (see Section 5.3), no effects on reproductive capacity were observed in male and female Wistar rats that were mated after 3 months of treatment 5 days/week with o-nitrotoluene at 200 mg/kg/day, m-nitrotoluene at 300 mg/kg/day or p-nitrotoluene at 400 mg/kg/day. In the same study, the adult rats were killed after 6 months of treatment. Gross and histological examination revealed testicular atrophy and necrosis of seminiferous tubules in male rats treated with p-nitrotoluene, but not o- or m-nitrotoluene.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

No chronic studies were located in the available literature; however, results of several subchronic studies indicate that exposure to the nitrotoluenes may result in hemolytic anemia and liver and kidney lesions.

In the 6-month study by Ciss et al. (1980), groups of 10 male and 10 female Wistar rats were treated by gavage with o- or m-nitrotoluene in olive oil or p-nitrotoluene in methyl cellulose, 5 days/week. The doses were 200 mg/kg/day for o-nitrotoluene, 300 mg/kg/day for m-nitrotoluene and 400 mg/kg/day for p-nitrotoluene. Similar groups of rats served as vehicle-controls. The only gross sign of toxicity was alopecia in females treated with m- or p-nitrotoluene; the hair loss was greater in the p-nitrotoluene group. No treatment-related effects were observed on growth, mortality or hematological parameters, with the exception of a slight decrease in the hemoglobin concentration of all nitrotoluene-treated groups. Sporadic

changes in serum enzyme concentrations were observed, but no consistent patterns could be discerned. Histological examination of the major organs of the rats killed after 6 months revealed unspecified lesions in the spleens of male rats treated with any of the three isomers. In rats treated with o-nitrotoluene, lesions and hyalin deposits in the renal tubules were observed. Testicular atrophy and necrosis of the seminiferous tubules occurred in the majority of the males treated with p-nitrotoluene.

Additional data regarding the effects of subchronic oral exposure to the three isomers of nitrotoluene were obtained from translations of two Russian reports (Kovalenko, 1973; Vasilenko and Kovalenko, 1975), which appear to be the same study. An unspecified number of white rats (sex not specified) were treated by gavage (vehicle not specified) with o-, m- or p-nitrotoluene at doses of 322, 318 or 392 mg/kg/day, respectively, daily for 30 days or 3 days/week for 3 months. Effects on hematological parameters were observed, which are suggestive, in part, of hemolytic anemia. Rats treated with any isomer for 30 days or for 3 months had a slight sulfhemoglobinemia; no significant differences among isomers or between exposure regimens were apparent. Methemoglobin concentrations were significantly increased compared with controls ($p<0.05$) in rats treated for 30 days with m- or p-nitrotoluene, but not o-nitrotoluene; methemoglobinemia was not observed in the 3-month experiment. All three isomers caused the appearance of Heinz bodies in the 3-month experiment; only p-nitrotoluene treatment resulted in Heinz body formation in the 30-day experiment. Rats treated with m- or p-nitrotoluene for 30 days also had decreased hemoglobin concentration and erythrocyte counts compared with controls; the decreases were accompanied by reticulocytosis and increased resistance of erythrocytes to acid hemolysis.

Treatment for 2-3 months with o- and m-nitrotoluene resulted in prolonged clotting time. Rats treated with p-nitrotoluene for an unspecified period of time had reduced body weight gains. No significant effects were observed on liver and kidney weights, liver function (measured by bromosulfophthalein retention time) or kidney function (indicated by blood and urine levels of urea).

In another subchronic study from the Russian literature, Kosachevskaya (1967) administered a mixture of the nitrotoluene isomers in water by gavage to groups of eight rats at 0, 240 or 480 mg/kg/day daily for 1.5-2 months. Gross signs of toxicity included restlessness, irritation of the snout, unstable gait, paresis of the hindlegs and lethargy, which were observed immediately after dosing. It was not clear from the translation if these signs were seen after administration of both doses or only after the high dose. Dose-related increased severity of hematological changes included decreased hemoglobin concentration, increased white blood cell count, decreased erythrocyte count; decreased percentage of eosinophils and monocytes and an increased percentage of lymphocytes, decreased prothrombin time, increased platelet count and increased methemoglobin concentration. Plethora and hemorrhaging occurred in some rats treated at both dose levels. Hemosiderosis of the spleen and fatty degeneration of the liver occurred in some rats treated with 480 mg/kg/day.

Information about human occupational exposure was available from abstracts (Nemtseev and Smolyaninova, 1976; Makotchenko, 1974) and a translation (Kleiner and Stovpivskaya, 1981) of Russian studies. Nemtseev and Smolyaninova (1976) conducted vision tests in persons working in contact with nitrotoluene (mixed isomers) and found evidence of toxic neuropathy of the optic pathway, representative of a "reduction in time and constriction of visual field." Makotchenko (1974) reported that chronic poisoning of

patients with nitrogen-containing toluene compounds resulted in dysfunction of the adrenal cortex.

Kleiner and Stovpivskaya (1981) performed clinical and laboratory tests on a group of 38 women (age 30-45 years) with >5 years occupational exposure to nitrogen-containing toluene compounds to study disturbances of intestinal digestion. Controls consisted of 24 healthy individuals (sex not specified) who had not been exposed occupationally to chemicals. Intestinal dyspepsia was a common finding in the exposed group: 83.9% had shooting pains around the navel; 79% had constipation; 47.4% had distention of the intestine, 41% had intolerance to milk, 15% had variable stools. Spastic dyskinesia of the small intestine was detected radiologically in ~1/3 of the subjects. Significant differences compared with controls included elevated enterokinase and alkaline phosphatase activities in the duodenal juice and feces, a decrease in intestinal amylase activity, an increase in adsorbed amylase and impaired absorption of carbohydrates and fats. The results of an experiment in which dogs were treated subcutaneously with trinitrotoluene supported the conclusion that nitrotoluene compounds were involved in the etiology of digestive disturbances.

5.6. OTHER RELEVANT INFORMATION

Values for the acute oral LD_{50} of o-nitrotoluene range from 890-1610 mg/kg for rats and 970-2460 mg/kg for mice (NIOSH, 1985; Vernot et al., 1977; Vasilenko and Kovalenko, 1975). For m-nitrotoluene, the acute oral LD_{50} values are 1072-1590 mg/kg for rats, 330-1230 mg/kg for mice and 3600 mg/kg for guinea pigs. For the p-isomer, the values range from 1960-2140 mg/kg for rats and 1231-1960 mg/kg for mice. Vasilenko and Kovalenko (1975) reported an acute oral LD_{50} for rabbits of 1750 mg/kg for each of the isomers. Because of the disparity in the reported values, it is difficult to make comparisons among species and isomers.

Kinkead et al. (1977) exposed mice and rats by inhalation to the highest attainable concentrations of the nitrotoluenes [354 and 320 ppm (1986 and 1795 mg/m^3), respectively, for o-nitrotoluene; 151 and 157 ppm (847 and 881 mg/m^3), respectively, for m-nitrotoluene; and 228 and 152 ppm (1279 and 853 mg/m^3), respectively, for p-nitrotoluene] for 4 hours. No deaths occurred in the 14-day observation period. There were no signs of toxicity and no gross lesions in the animals that were killed after 14 days.

NIOSH (1985) reported a dermal LD_{50} in rats of 16 g/kg for m-nitrotoluene; however, Kinkead et al. (1977) observed no deaths or signs of toxicity in rabbits dermally exposed to undiluted o-, m- or p-nitrotoluene (20 g/kg) under cover for 24 hours. The nitrotoluene isomers were also not irritating to the intact or abraded skin of rabbits.

The nitrotoluenes are considered to be moderately toxic to humans; ingestion of 1 oz to 1 pt may cause death (OHM/TADS, 1985).

5.7. SUMMARY

Carcinogenicity testing by the National Toxicology Program of p-nitrotoluene by gavage treatment of rats and mice is in progress; the o- and m-isomers are not scheduled for testing (NTP, 1985). No significant differences in the incidence of skin papillomas or carcinomas were observed in mice treated topically with single applications of o-nitrotoluene (24-240 mg/mouse) or p-nitrotoluene (50-400 mg/mouse) followed by 30 weekly applications of TPA as a promotor, when compared with mice treated with TPA alone (Slaga et al., 1985). Equivocal results were obtained in a pulmonary adenoma assay, in which mice were injected intraperitoneally with o-nitrotoluene (840-4800 mg/kg) or p-nitrotoluene (1800-9000 mg/kg), 3 days/week for 8 weeks (Slaga et al., 1985). Therefore, the data are insufficient to conclude that the nitrotoluenes are carcinogenic.

The nitrotoluenes have been tested for mutagenicity and genotoxicity with different responses depending upon the isomer tested (see Table 5-1). In general, m-nitrotoluene was negative for reverse mutation in Salmonella typhimurium with and without metabolic activation and in assays for UDS, chromosome aberrations and SCE in mammalian systems. For p-nitrotoluene, equivocal results were obtained in reverse mutation assays, but positive results were obtained in assays for mitotic gene conversion in Saccharomyces cerevisiae and SCE and chromosome aberrations in mammalian cells. o-Nitrotoluene was negative for reverse mutation in S. typhimurium with and without S-9 and for chromosome effects and UDS in vitro; however, Doolittle et al. (1983) presented evidence that intestinal microflora played a role in the bioactivation of o-nitrotoluene to a metabolite capable of damaging liver DNA.

No malformed offspring resulted from matings of male and female rats that were treated by gavage with 200 mg/kg/day o-nitrotoluene, 300 mg/kg/day m-nitrotoluene or 400 mg/kg/day p-nitrotoluene, 5 days/week for 3 months before mating and throughout gestation (Ciss et al., 1980). Slight histological lesions, however, were observed in the spleens of the offspring of rats exposed to m-nitrotoluene. Reproductive capacity of the adult rats was not impaired at 3 months; however, after 6 months of treatment with p-nitrotoluene, male rats had histological evidence of testicular atrophy and necrosis of seminiferous tubules.

Subchronic studies in rats indicate that exposure to nitrotoluenes results in kidney and liver lesions and evidence of hemolytic anemia with subsequent increased erythropoiesis. In the study by Ciss et al. (1980), rats treated with m- or p-nitrotoluene had alopecia; rats treated with any of the isomers had lesions in the spleen; and rats treated with o-nitrotoluene had hyalin deposits and lesions in the renal tubules. Treatment of

rats with 322 mg/kg/day o-nitrotoluene, 318 mg/kg/day m-nitrotoluene or 392 mg/kg/day p-nitrotoluene daily for 30 days or 3 days/week for 3 months resulted in hematological effects suggestive of hemolytic anemia (Kovalenko, 1973; Vasilenko and Kovalenko, 1975). Sulfhemoglobinemia was a common finding for all isomers and treatment regimens. Methemoglobinemia, reduced hemoglobin concentrations, decreased erythrocyte counts, increased reticulocyte counts, decreased percentage of monocytes and eosinophils, reduced percentage lymphocytes, Heinz body formation and prolonged clotting time were observed depending upon the isomer administered and the treatment regimen. Treatment of rats by gavage with a mixture of isomers at 480 mg/kg/day and possibly 240 mg/kg/day for 1.5-2 months resulted in signs of CNS toxicity immediately after dosing (Kosachevskaya, 1967). The severity of hematological changes increased with dose. Some rats treated with 480 mg/kg/day had hemosiderosis of the spleen and fatty degeneration of the liver.

Occupational exposure to nitrotoluenes has been associated with optic neuropathy (Nemtseev and Smolyaninova, 1976), dysfunction of the adrenal cortex (Makotchenko, 1974) and disturbances of intestinal digestion (Kleiner and Stovpivskaya, 1981). These effects were not correlated with exposure levels. Ingestion of 1 oz to 1 pt of the nitrotoluenes may be fatal to humans (OHM/TADS, 1985).

The acute oral LD_{50} values for the nitrotoluenes in rats range from 890-1610 mg/kg/day for o-nitrotoluene, 1072-1590 mg/kg for m-nitrotoluene and 1960-2140 mg/kg for p-nitrotoluene (NIOSH, 1985; Vernot et al., 1977; Vasilenko and Kovalenko, 1975).

6. AQUATIC TOXICITY

6.1. ACUTE

Studies comparing the toxicity of nitrotoluene isomers to various aquatic species are summarized in Table 6-1. The three isomers are about equally toxic to minnows, with m-nitrotoluene being slightly more toxic than the others. Using a static bioassay, Liu et al. (1983) determined 96-hour LC_{50} values for fathead minnows, Pimephales promelas, of 37.1, 32.5 and 49.7 mg/ℓ for o-, m- and p-nitrotoluene, respectively. McKee and Wolf (1963) reported a 96-hour LC_{50} of 30 mg/ℓ m-nitrotoluene for fathead minnows. According to Mattson et al. (1976), the 1-hour LC_{50} of m-nitrotoluene to fathead minnows was 43 mg/ℓ, and the 24- to 96-hour LC_{50} was 30 mg/ℓ. The LC_{50} of o-nitrotoluene to the golden orfe, Leuciscus idus melanotus, was 29 mg/ℓ (LC_0=11 mg/ℓ, LC_{100}=57 mg/ℓ) (Juhnke and Luedemann, 1978).

Daphnia magna is apparently more sensitive to p-nitrotoluene than to the other isomers (see Table 6-1). The 24-hour LC_{50} values for o-, m- and p-nitrotoluene were 42, 35 and 11 mg/ℓ, respectively (Bringmann and Kuehn, 1977). The EC_{50}s for immobilization of Daphnia magna were 16, 24 and 9 mg/ℓ for o-, m- and p-nitrotoluene, respectively (Bringmann and Kuehn, 1982). For Daphna magna, Liu et al. (1983) reported EC_{50} values of >77.1, 28.1 and 11.8 mg/ℓ for o-, m- and p-nitrotoluene, respectively.

No clear trends in isomer toxicity were evident in tests with protozoans (see Table 6-1). Toxicity thresholds for inhibition of cell multiplication ranged from 12-48 mg/ℓ (Bringmann and Kuehn, 1981).

6.2. CHRONIC

Sloof and Canton (1983) determined subchronic NOECs for different effects of p-nitrotoluene on several aquatic species (Table 6-2). The

TABLE 6-1

Comparative Toxicity of Nitrotoluene Isomers to Aquatic Organisms

Species	Parameter	Isomer (mg/ℓ)			Reference
		o-isomer	m-isomer	p-isomer	
Minnows	minimum lethal concentration	35-40	25-30	45-50	McKee and Wolf, 1963
Fathead minnow *Pimephales promelas*	96-hour LC_{50}	37.1	32.5	49.7	Liu et al., 1983
Water flea *Daphnia magna*	24-hour LC_{50} (LC_0; LC_{100})	42 (13;100)	35 (11;88)	11 (7;20)	Bringmann and Kuehn, 1977
Water flea *Daphnia magna*	EC_{50} (immobilization) (EC_0; EC_{100})	16 (6.8;26)	24 (13;51)	9 (5.6;12)	Bringmann and Kuehn, 1982
Water flea *Daphnia magna*	48-hour EC_{50}	>77.1	28.1	11.8	Liu et al., 1983
Ciliate protozoan *Uronema parduczi*	toxicity threshold	24	24	46	Bringmann and Kuehn, 1981
Flagellate protozoan *Chilomonas paramaecium*	toxicity threshold	27	48	16	Bringmann and Kuehn, 1981
Flagellate protozoan *Entosiphon sulcatum*	toxicity threshold	46	12	8.6	Bringmann and Kuehn, 1981
Green alga *Scenedesmus quadricauda*	toxicity threshold	28	4.4	15	Bringmann and Kuehn, 1978
Blue-green alga *Microcystis aeruginosa*	toxicity threshold	3.1	1.0	3.3	Bringmann and Kuehn, 1978
Bacteria *Pseudomonas putida*	toxicity threshold	18	10	26	Bringmann and Kuehn, 1976

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TABLE 6-2

NOEC Values for Various Effects of p-Nitrotoluene in Different Aquatic Organisms[a]

Species	Exposure Time (days)	Number of Organisms per Group	Dosing[b]	Criteria	NOEC (mg/L)
Bacteria Pseudomonas fluorescens	0.3	10^{10}	static	specific growth rate	10.0
Blue-green alga Microcystis aeruginosa	4	1.5×10^6	static	specific growth rate	3.2
Green alga Scendesmus pannonicus	4	1.5×10^6	static	growth (biomass)	10.0
Duckweed Lemna minor	7	2	static	growth (biomass)	10.0
Water flea Daphnia magna	21	25	semi-static	mortality reproduction	3.2 1.0
Mosquito Culex pipiens	25	30	semi-static	mortality development	3.2 3.2
Hydra Hydra oligactis	21	2	semi-static	specific growth rate	10.0
Snail Lymnaea stagnalis	40	20	semi-static	mortality reproduction	10.0 0.32
Snail Lymnaea stagnalis (egg capsules)	7	5	semi-static	hatching	10.0
Guppy Poecilia reticulata	28	25	semi-static	mortality mortality and behavior growth	10.0 10.0 10.0
Medaka Oryzias latipes (eggs)	40	35	semi-static	mortality mortality and behavior hatching growth	1.0 1.0 32.0
Tadpoles Xenopus laevis	100	75	semi-static	mortality development growth	10.0 3.2 32.0

[a]Source: Sloof and Canton, 1983

[b]Semi-static -- water was changed every Monday, Wednesday and Friday.

highest NOECs for mortality, behavior and growth were all 10 mg/ℓ for guppies, Poecilia reticulata, exposed for 28 days. For medakas, Oryzias latipes, 40-day NOECs for mortality and behavior were both 1.0 mg/ℓ. The most sensitive effect in tadpoles, Xenopus laevis, was inhibition of development, having a NOEC of 3.2 mg/ℓ.

Among invertebrates, reproduction in the snail, Lymnaea stagnalis, was the most sensitive parameter measured, with a NOEC of 0.32 mg/ℓ. Daphnia magna had NOECs for mortality and reproduction of 3.2 and 1.0 mg/ℓ, respectively. For the mosquito, Culex pipiens (larvae), the NOEC for mortality and developmental effects was 3.2 mg/ℓ (Sloof and Canton, 1983).

6.3. PLANTS

The toxicity thresholds for inhibition of cell multiplication of a blue-green alga, Microcystis aeruginosa, a green alga, Scenedesmus quadricauda, and bacteria, Pseudomonas putida, determined by Bringmann and Kuehn (1976, 1978) (see Table 6-1) agree with NOECs for similar species determined by Sloof and Canton (1983) (see Table 6-2). Of the three isomers, m-nitrotoluene was the most toxic to the algae and bacteria (see Table 6-1). The blue-green alga, Microcystis aeruginosa, was more sensitive to nitrotoluene toxicity (toxicity threshold=1.0-3.3 mg/ℓ; NOEC=3.2 mg/ℓ) than the green algae, Scenedesmus (toxicity threshold=4.4-28 mg/ℓ; NOEC=10 mg/ℓ) or the bacteria, Pseudomonas (toxicity threshold=10-26 mg/ℓ; NOEC = 10 mg/ℓ) (see Tables 6-1 and 6-2).

Sloof and Canton (1983) reported a NOEC of 10 mg/ℓ p-nitrotoluene for inhibition of growth of Lemna minor. In a study described by TSCA-ITC (1981), Lemna perpusilla colony growth was inhibited by 100 mg/ℓ o-nitrotoluene at pH 6.3 but not at pH 8.5. No effects occurred at either pH at 50, 10, 1 or 0.1 mg/ℓ.

6.4. RESIDUES

Liu et al. (1983) calculated BCFs from estimated K_{ow}s. The BCFs for o- and p-nitrotoluene were 32.96 and 39.26, respectively. Landrum and Crosby (1981) investigated the kinetics of an injected dose of p-nitrotoluene in the sea urchin, Strongylocentrotus purpuratus. The elimination rate constant was 0.3 $hour^{-1}$, and 52% of the total dose was eliminated in 8 hours. The p-nitrotoluene was transformed by reduction to p-toluidine and subsequent acetylation of the amine.

6.5. SUMMARY

There is no large variation in the toxicity of the nitrotoluene isomers, but m-nitrotoluene appears to be the most toxic to fish, algae and bacteria, while p-nitrotoluene is the most toxic to Daphnia (see Table 6-1). Snails, Lymnaea stagnalis, and Daphnia magna are especially sensitive to the reproductive effects of p-nitrotoluene, with NOECs of $\leq$1.0 mg/ℓ (see Table 6-2). Eggs of the medaka (fish), Oryzias latipes, are also sensitive to p-nitrotoluene, with a NOEC of 1.0 mg/ℓ for mortality (see Table 6-2). The blue-green alga, Microcystis aeruginosa, is the aquatic plant species most sensitive to nitrotoluene toxicity in general, having toxicity thresholds of 1.0-3.3 mg/ℓ for the three isomers (see Table 6-1).

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

The ACGIH (1985) has adopted a TWA-TLV for nitrotoluenes (all isomers) of 2 ppm (11 mg/m^3) with a "skin" notation, which indicates that exposure by the dermal route may contribute to the overall exposure. The TLV was based on analogy to aniline (ACGIH, 1980).

The OSHA standard for occupational exposure to nitrotoluene (all isomers) is 5 ppm (~30 mg/m^3) (Code of Federal Regulations, 1983).

7.2. AQUATIC

Guidelines and standards for the protection of aquatic organisms from the toxic effects of the various nitrotoluenes were not located in the available literature cited in the Appendix.

8. RISK ASSESSMENT

Carcinogenicity testing by the National Toxicology Program of p-nitrotoluene by gavage treatment of rats and mice is in progress; the o- and m-isomers are not scheduled for testing (NTP, 1985). No significant differences in the incidence of skin papillomas or carcinomas were observed in mice treated topically with single applications of o-nitrotoluene (24-240 mg/mouse) or p-nitrotoluene (50-400 mg/mouse) followed by 30 weekly applications of TPA as a promotor, when compared with mice treated with TPA alone (Slaga et al., 1985). Equivocal results were obtained in a pulmonary adenoma assay, in which mice were injected intraperitoneally with o-nitrotoluene (840-4800 mg/kg) or p-nitrotoluene (1800-9000 mg/kg), 3 days/week for 8 weeks (Slaga et al., 1985). Therefore, the data are insufficient to conclude that the nitrotoluenes are carcinogenic.

The nitrotoluenes have been tested for mutagenicity and genotoxicity with different responses depending upon the isomer tested (see Table 5-1). In general, m-nitrotoluene was negative for reverse mutation in Salmonella typhimurium with and without metabolic activation and in assays for UDS, chromosome aberrations and SCE in mammalian systems. For p-nitrotoluene, equivocal results were obtained in reverse mutation assays, but positive results were obtained in assays for mitotic gene conversion in Saccharomyces cerevisiae and SCE and chromosome aberrations in mammalian cells. o-Nitrotoluene was negative for reverse mutation in S. typhimurium with and without S-9 and for chromosome effects and UDS in vitro; however, Doolittle et al. (1983) presented evidence that intestinal microflora played a role in the bioactivation of o-nitrotoluene to a metabolite capable of damaging liver DNA.

No malformed offspring resulted from matings of male and female rats that had been treated by gavage with 200 mg/kg/day o-nitrotoluene, 300 mg/kg/day m-nitrotoluene or 400 mg/kg/day p-nitrotoluene, 5 days/week for 3 months before mating and throughout gestation (Ciss et al., 1980). Slight histological lesions, however, were observed in the spleens of the offspring of rats exposed to m-nitrotoluene. Reproductive capacity of the adult rats was not impaired at 3 months; however, after 6 months of treatment with p-nitrotoluene, male rats had histological evidence of testicular atrophy and necrosis of seminiferous tubules.

Results of subchronic studies indicate that exposure to nitrotoluenes results in kidney and liver lesions and evidence of hemolytic anemia with subsequent increased erythropoiesis. In the study by Ciss et al. (1980), rats treated with m- or p-nitrotoluene had alopecia, rats treated with any of the isomers had lesions in the spleen and rats treated with o-nitrotoluene had hyalin deposits and lesions in the renal tubules. Treatment of rats with 322 mg/kg/day o-nitrotoluene, 318 mg/kg/day m-nitrotoluene or 392 mg/kg/day p-nitrotoluene daily for 30 days or 3 days/week for 3 months resulted in hematological effects suggestive of hemolytic anemia (Kovalenko, 1973; Vasilenko and Kovalenko, 1975). Sulfhemoglobinemia was a common finding for all isomers and treatment regimens. Methemoglobinemia, reduced hemoglobin concentrations, decreased erythrocyte counts, increased reticulocyte counts, decreased percentage of monocytes and eosinophils, decreased percentage of lymphocytes, Heinz body formation and prolonged clotting time were observed depending upon the isomer administered and the treatment regimen. Treatment of rats by gavage with a mixture of isomers at 480 mg/kg/day and possibly 240 mg/kg/day for 1.5-2 months resulted in signs of CNS toxicity immediately after dosing (Kosachevskaya, 1967). The severity

of hematological changes increased with dose. Some rats treated with 480 mg/kg/day had hemosiderosis of the spleen and fatty degeneration of the liver.

Occupational exposure to nitrotoluenes has been associated with optic neuropathy (Nemtseev and Smolyaninova, 1976), dysfunction of the adrenal cortex (Makotchenko, 1974) and disturbances of intestinal digestion (Kleiner and Stovpivskaya, 1981). These effects were not correlated with exposure levels. Ingestion of 1 oz to 1 pt of the nitrotoluenes may be fatal to humans (OHM/TADS, 1985).

Since data are insufficient at this time to conclude that the nitrotoluenes are carcinogenic, it is appropriate to derive an ADI based on toxicity data.

The studies of Kovalenko (1973) and Vasilenko and Kovalenko (1975) are flawed due to the deficiencies in experimental design description, results presentation and absence of histopathological evaluations. The 90-day study of this series additionally utilized a dosing regimen of only 3 days/week. A NOEL or NOAEL was not identified in these studies which utilized doses of 322, 318 and 329 mg/kg/day for o-, m- and p-nitrotoluene, respectively. The doses of o-, m- and p-nitrotoluene of 200, 300 and 400 mg/kg/day, respectively, 5 days/week in the study by Ciss et al. (1980) are equivalent to transformed doses of 143, 214 and 286 mg/kg/day, respectively. The dose of mixed isomers of 240 mg/kg/day in the study of Kosachevskaya (1967) was apparently administered 7 days/week.

None of the subchronic studies identify doses which correspond to NOELs or NOAELs. The study reported in most detail is Ciss et al. (1980). However, this study only offered a limited description of histopathological findings making it difficult to assess severity. In this study all three isomers produced lesions in the spleens of male rats treated for 6 months.

In addition, pups from parental animals treated with the m-isomer exhibited splenic lesions. Rats treated with the o-isomer exhibited lesions and hyalin deposits in the renal tubules while males treated with the p-isomer showed atrophy of the testes and necrosis of the seminiferous tubules. Although these effects sound more severe than effects reported by Kovalenko (1973) and Vasilenko and Kovalenko (1975) which included sulfhemaglobinemia, Heinz body formation and prolonged clotting time, these latter two studies did not report histopathological evaluations.

The overall data base is weak. The most appropriate basis for ADI development is Ciss et al. (1980). In this study transformed doses of 143, 214 and 286 mg/kg of the o-, m- and p-isomers, respectively, were administered. It would be preferable to develop isomer specific ADI estimates. However, since only one dose was used for each isomer, since effects were produced following treatment with each and since lesions of the spleen were common to all three groups, it is proposed to utilize the lowest LOAEL, 143 mg/kg/day, as the basis for all three isomers. Dividing the LOAEL of 143 mg/kg/day by an uncertainty factor of 10,000 (10 for the uncertainty associated with an effect level, 10 for extrapolation based on a subchronic study, 10 for interspecies extrapolation and 10 to protect sensitive subgroups) results in a provisional ADI of 0.0143 mg/kg/day or 1 mg/day for a 70 kg human. This ADI is considered a provisional estimate for all three isomers based on a limited data base and should be reevaluated when more complete data are available.

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

Occupational exposure of humans to nitrotoluenes has been associated with optic neuropathy (Nemtseev and Smolyaninova, 1976), dysfunction of the adrenal cortex (Makotchenko, 1974) and disturbances of intestinal digestion (Kleiner and Stovpivskaya, 1981). Since exposure levels were not determined, these studies cannot be used in the derivation of the RQ.

No studies of chronic duration in animals were located; however, several subchronic oral studies in rats were available for consideration in deriving an RQ for nitrotoluenes. These studies were discussed in Chapter 5 and are summarized in Table 9-1. As seen from Table 9-1, the effects observed after exposure to the lowest adjusted doses of any of the nitrotoluenes included evidence of hemolytic anemia and increased erythropoiesis (decreased hemoglobin concentration, decreased erythrocyte counts, reticulocytosis, Heinz body formation, hemosiderosis in the spleen). At slightly higher doses lesions of the spleen, kidney and testes were noted. The lowest transformed doses at which evidence of hemolytic anemia was observed were 138, 136 and 168 mg/kg/day for o-, m- and p-nitrotoluene, respectively, in the study by Kovalenko (1973) and Vasilenko and Kovalenko (1975). The lowest transformed dose associated with histopathological lesions was 143 mg/kg/day o-nitrotoluene (Ciss et al., 1980). m-Nitrotoluene resulted in lesions in the spleens of offspring of animals treated with doses of 214 mg/kg. Since these effects were observed for all three isomers at similar doses, it is appropriate to calculate a CS and RQ for all three isomers from the lowest dose, 136 mg/kg/day. Multiplying 136 mg/kg/day by the cube root of the ratio of the rat body weight (assumed to be 0.35 kg) to the reference human body weight (70 kg) and by 70 kg results in an equivalent human dose of 1628 mg/day. Because the study was subchronic, it is appropriate to

TABLE 9-1

Oral Toxicity Summary for Nitrotoluenes

Compound	Species/ Strain	Sex	No. at Start	Weight	Vehicle	Purity	Dosage/ Exposure (mg/kg/day)	Transformed Dose (mg/kg/day)	Response	Reference
o-Nitro-toluene	rat/NR	NR	NR	NR	NR	NR	322 daily for 30 days	322	Sulfhemoglobinemia	Kovalenko, 1973; Vasilenko and Kovalenko, 1975
o-Nitro-toluene	rat/NR	NR	NR	NR	NR	NR	322 3 days/ week for 3 months	138	Sulfhemoglobinemia, prolonged clotting time, Heinz body formation	Kovalenko, 1973; Vasilenko and Kovalenko, 1975
o-Nitro-toluene	rat/ Wistar	M,F	10/sex	200 g	olive oil	99%	200 5 days/ week for 6 months	143	Slight decrease in hemoglobin concentration, lesions in spleen and renal tubules, hyalin deposits, alopecia	Ciss et al., 1980
m-Nitro-toluene	rat/NR	NR	NR	NR	NR	NR	318 daily for 30 days	318	Sulfhemoglobinemia, methemoglobinemia, decreased hemoglobin concentration, decreased erythrocyte count, increased reticulocyte count	Kovalenko, 1973; Vasilenko and Kovalenko, 1975
m-Nitro-toluene	rat/NR	NR	NR	NR	NR	NR	318 3 days/ week for 3 months	136	Sulfhemoglobinemia, Heinz body formation, prolonged clotting time	Kovalenko, 1973; Vasilenko and Kovalenko, 1975
m-Nitro-toluene	rat/ Wistar	M,F	10/sex	200 g	olive oil	99%	300 5 days/ week for 6 months	214	Slight decrease in hemoglobin concentration, alopecia, lesions in spleen of adult (at 6 months) and offspring (at 3 months)	Ciss et al., 1980
p-Nitro-toluene	rat/NR	NR	NR	NR	NR	NR	392 daily for 30 days	392	Sulfhemoglobinemia, methemoglobinemia, decreased hemoglobin concentration, decreased erythrocyte count, increased reticulocyte count, decreased resistance of erythrocytes to acid hemolysis	Kovalenko, 1973; Vasilenko and Kovalenko, 1975
p-Nitro-toluene	rat/NR	NR	NR	NR	NR	NR	392 3 days/ week for 3 months	168	Sulfhemoglobinemia, Heinz body formation, reduced body weight	Kovalenko, 1973; Vasilenko and Kovalenko, 1975

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TABLE 9-1 (cont.)

Compound	Species/ Strain	Sex	No. at Start	Weight	Vehicle	Purity	Dosage/ Exposure (mg/kg/day)	Transformed Dose (mg/kg/day)	Response	Reference
p-Nitro-toluene	rat/ Wistar	M,F	10/sex	200 g	methyl cellulose	99%	400 5 days/ week	286	Decreased hemoglobin concentration, spleen lesions, testicular atrophy and necrosis of seminiferous tubules	Ciss et al., 1980
Mixed isomers	rat/NR	NR	8/group	NR	water	NR	240 and 480 daily for 1.5-2 months	240 and 480	Gross signs of CNS toxicity immediately after dosing with high dose; dose-related decreases in hemoglobin concentration, erythrocyte count, prothrombin times; dose-related increase in leukocyte counts, changes in differential leukocyte count, methemoglobinemia; plethora and hemorrhaging, hemosiderosis of spleen and fatty degeneration of liver at 480 mg/kg/day	Kosachevskaya, 1967

NR = Not reported

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divide this equivalent human dose by an uncertainty factor of 10, which results in a chronic MED of 162.8 mg/day (Table 9-2). The MED corresponds to an RV_d of 2.2. The effect of hemolytic anemia warrants an RV_e of 5. Multiplying the RV_d by the RV_e results in a CS of 11.0.

Exposure of adult male and female rats to the nitrotoluenes for 3 months before mating and during gestation did not result in malformed offspring (Ciss et al., 1980). Lesions in the spleen of the offspring of the m-nitrotoluene exposed adults were observed, however, and may be considered manifestations of fetotoxicity, which warrants an RV_e of 8. Multiplying the transformed dose of 214 mg/kg/day by the cube root of the body weight ratio and by 70 kg results in an MED of 2562 mg/day. The MED corresponds to an RV_d of 1. Multiplying the RV_d by the RV_e results in a CS of 8, corresponding to an RQ of 1000 (see Table 9-2). Similarly, a transformed dose of 171.2 mg/kg/day o-nitrotoluene which resulted in spleen and kidney lesions corresponds to an RV_d of 2.2, an RV_e of 7 and a CS of 15.1 (Ciss et al., 1980). All of the composite scores correspond to RQs of 1000 lbs.

In the study of Kosachevskaya (1967), signs of CNS toxicity were observed immediately after gavage dosing of rats with a mixture of the isomers at 480 mg/kg; therefore, the effects were acute responses and cannot be used for RQ derivation. The liver lesions observed in these rats warrant an RV_e of 5 or 6. Since the effects were observed at higher doses than the evidence of hemolytic anemia, no CS for these effects is shown on Table 9-2.

Examination of Table 9-2 reveals that all CSs correspond to an RQ of 1000; however, the CS for spleen and kidney lesions is the highest. Therefore, the study of Ciss et al. (1980) is recommended as the basis for the RQ for the nitrotoluenes (Table 9-3). When results of the NTP (1985) study become available, they may provide a better basis for the RQ classification.

TABLE 9-2

Oral Composite Scores for Nitrotoluenes

Compound	Species	Animal Dose (mg/kg/day)	Chronic Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
o-, m- or p-Nitrotoluene	rat	136	162.8*	2.2	evidence of hemolytic anemia	5	11.0	1000	Kovalenko, 1973; Vasilenko and Kovalenko, 1975
m-Nitrotoluene	rat	214	2562	1	fetotoxicity	8	8	1000	Ciss et al., 1980
o-Nitrotoluene	rat	143	171.2*	2.1	spleen and kidney lesions	7	15.1	1000	Ciss et al., 1980

*The dose was divided by an uncertainty factor of 10 to approximate chronic exposure.

TABLE 9-3

Nitrotoluenes

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	171.2 mg/day
Effect:	spleen and kidney lesions
Reference:	Ciss et al., 1980
RV_d:	2.1
RV_e:	7
Composite Score:	15.1
RQ:	1000

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*Equivalent human dose

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

Carcinogenicity testing by the National Toxicology Program of p-nitrotoluene by gavage treatment of rats and mice is in progress; the o- and m-isomers are not scheduled for testing (NTP, 1985).

An unspecified number of SENCAR mice were treated topically with single applications of 24, 120 or 240 mg of o-nitrotoluene in acetone or 50, 250 or 400 mg of p-nitrotoluene in acetone (Slaga et al., 1985). Treatment with the nitrotoluenes was followed by 30 weekly applications of 4 µg TPA. p-Nitrotoluene was negative as a tumor initiator. Higher incidences of skin papillomas and carcinomas were observed in the mice initiated with o-nitrotoluene compared with mice treated with TPA alone; however, the differences were not significant. Slaga et al. (1985) concluded that o-nitrotoluene might be a weak skin tumor initiator.

Equivocal results were obtained in a pulmonary adenoma assay in mice (Slaga et al., 1985). Groups of 30 A/Jax male mice were treated intraperitoneally with o-nitrotoluene at 840, 2100 or 4800 mg/kg or p-nitrotoluene at 1800, 4500 or 9000 mg/kg, 3 days/week for 8 weeks and observed for an additional 16 weeks before sacrifice. The percentage of survivors with lung tumors and the number of tumors/lung increased in a dose-related manner for both isomers; however, no significant differences were found between nitrotoluene-treated mice, corn oil-treated controls or untreated controls. It was concluded that the nitrotoluenes did not give "an unequivocally positive response."

No other carcinogenicity studies in animals or humans were located in the available literature; therefore, pending the results of the NTP (1985) study of p-nitrotoluene, no potency factor can be derived.

Since data are insufficient at this time to indicate that the nitrotoluenes are carcinogenic, these compounds are classified as IARC Group 3 chemicals. Based on EPA's proposed guidelines, the nitrotoluenes are classified as Group D, not classifiable as to human carcinogenicity (U.S. EPA, 1984).

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APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CA SEARCH (Files 308, 309, 310, 311, 320)
TOXLINE
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP

These searches were conducted in August, 1985 with the exception of the TOXLINE, which was searched in February, 1984 and was kindly supplied by Dynamac Corp. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

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Windholz, M., Ed. 1983. The Merck Index, 10th ed. Merck and Co., Inc., Rahway, NJ.

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

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Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. USDI, Fish and Wildlife Serv. Res. Publ. 137, Washington, DC.

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